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DIAMINOACID-AMINOACID-POLYAMINE BASED GEMINI SURFACTANT COMPOUNDS

This application claims the benefit of UK priority application No. GB0207283.3 filed 27 March 2002 and GB0213646.3 filed 13 June 2002, whose contents are incorporated herein by reference.

This invention relates to newly identified diaminoacid-polyamine:peptide and diaminoacid-aminoacid-polyamine based gemini surfactant compounds, to the use of such compounds and to their production. The invention also relates to the use of the diaminoacid-polyamine:peptide based gemini compounds to facilitate the transfer of compounds into cells for drug delivery.

Surfactants are substances that markedly affect the surface properties of a liquid, even at low concentrations. For example surfactants will significantly reduce surface tension when dissolved in water or aqueous solutions and will reduce interfacial tension between two liquids or a liquid and a solid. This property of surfactant molecules has been widely exploited in industry, particularly in the detergent and oil industries. In the 1970s a new class of surfactant molecule was reported, characterised by two hydrophobic chains with polar heads which are linked by a hydrophobic bridge (Deinega,Y *et al.*, *Kolloidn. Zh.* 36, 649, 1974). These molecules, which have been termed "gemini" (Menger, FM and Littau,CA, *J.Am.Chem.Soc.* 113, 1451, 1991), have very desirable properties over their monomeric equivalents. For example they are highly effective in reducing interfacial tension between oil and water based liquids and have a very low critical micelle concentration (Menger, FM and Keiper, JS, *Angewandte. Chem. Int. Ed. Engl.*, 2000, 39, 1906).

Cationic surfactants have been used *inter alia* for the transfection of polynucleotides into cells in culture, and there are examples of such agents available commercially to scientists involved in genetic technologies (for example the reagent Tfx™-50 for the transfection of eukaryotic cells available from Promega Corp. WI, USA).

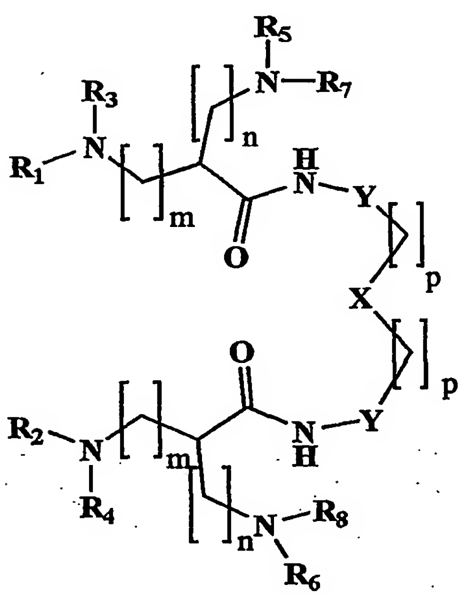
The efficient delivery of DNA to cells *in vivo*, either for gene therapy or for antisense therapy, has been a major goal for some years. Much attention has concentrated on the use of viruses as delivery vehicles, for example adenoviruses for epithelial cells in the respiratory tract with a view to corrective gene therapy for cystic fibrosis (CF). However, despite some evidence of successful gene transfer in CF patients, the adenovirus route remains problematic due to inflammatory side-effects and limited transient expression of the transferred gene. Several alternative methods for *in vivo* gene delivery have been investigated, including studies using cationic surfactants. Gao,X *et al. Gene Ther.* 2, 710-722,1995 demonstrated the feasibility of this approach with a normal human gene for CF transmembrane conductance regulator (CFTR) into the

respiratory epithelium of CF mice using amine carrying cationic lipids. This group followed up with a liposomal CF gene therapy trial which, although only partially successful, demonstrated the potential for this approach in humans (Caplen, NJ. *et al., Nature Medicine*, 1, 39-46, 1995). More recently other groups have investigated the potential of other cationic lipids for gene delivery (Miller, A, *Angew. Int. Ed. Engl.*, 37, 1768-1785, 1998), for example cholesterol derivatives (Oudrhiri,N et al. *Proc.Natl.Acad.Sci.* 94, 1651-1656, 1997). This limited study demonstrated the ability of these cholesterol based compounds to facilitate the transfer of genes into epithelial cells both *in vitro* and *in vivo*, thereby lending support to the validity of this general approach.

These studies, and others, show that in this new field of research there is a continuing need to develop novel low-toxicity surfactant molecules to facilitate the effective transfer of polynucleotides into cells both *in vitro* for transfection in cell-based experimentation and *in vivo* for gene therapy and antisense treatments. Gemini surfactants based on cysteine (WO99/29712) or on spermine (WO00/77032) or diamine (WO00/76954) have previously been made. Other examples of gemini surfactants are found in WO00/27795, WO02/30957 and WO02/50100.

The present invention seeks to overcome the difficulties exhibited by existing compounds.

The invention relates to diaminoacid-polyamine:peptide based gemini compounds having a diaminoacid-polyamine or a diaminoacid-aminoacid-polyamine backbone and conforming to the general structure of formula (I):

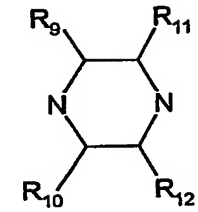


(I)

where:

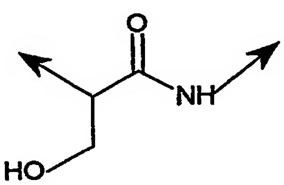
m = 0 to 6;
n = 0 to 7;
p = 0 to 6; and where

X = a bond, CH_2, $(CH_2)_2$, $NH(CH_2)qNH$ where q = 2 to 6, or



where R_9 to R_{12}, which can be the same or different, are selected from H, O or C_rH_{2r+1}, where r = 0 to 6 with the proviso that when R_9 and R_{12} are O, or when R_9 and R_{11} are O, then R_{10} and R_{11} or R_{10} and R_{12}, respectively, are H; and where

Y = a bond, CH_2,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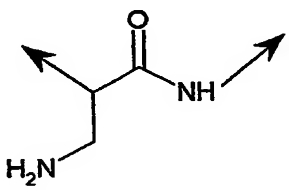
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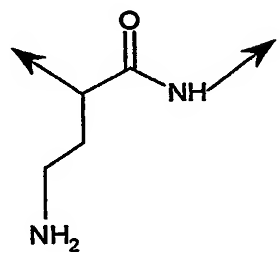
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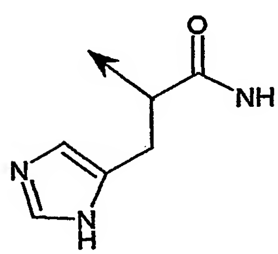
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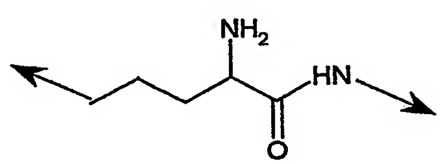
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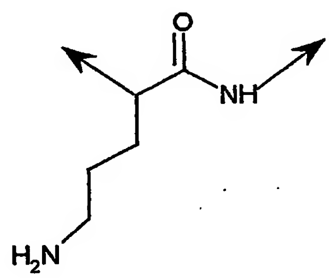

;

and where R_3, R_4, R_5, R_6, R_7 and R_8 are hydrogen and R_1 and R_2 are saturated or unsaturated hydrocarboxyl groups having up to 24 carbon atoms and linked to the diaminoacid-polyamine backbone by an amide bond;

or

where R_3, R_4, R_5 and R_6 are hydrogen, R_1 and R_2 are saturated or unsaturated hydrocarboxyl groups having up to 24 carbon atoms and linked to the diaminoacid-polyamine backbone by an amide bond, and where R_7 and R_8, which may be the same or different, are peptide groups formed from one or more amino acids linked

together by amide (CONH) bonds and further linked to the diaminoacid-polyamine backbone by amide bonds, in a linear or branched manner, having the general formula (II):

$$\begin{array}{l} -(A1)_{p1}-(A2)_{p2}-(A3)_{p3} \\ \qquad\qquad\quad | \\ \qquad\qquad (A4)_{p4} \end{array} \qquad (II)$$

where the values for p1 and p2, which may be the same or different, are from 0 to 5, preferably 1; and the values for p3 and p4, which may be the same or different, are from 0 to 5, preferably 0; A1, A3 and A4, which may be the same or different, is an amino acid selected from serine, lysine, ornithine, threonine, histidine, cysteine, arginine and tyrosine; and

A2 is an amino acid selected from lysine, ornithine and histidine;

or

a salt, preferably a pharmaceutically acceptable salt thereof.

Preferably, the compound is symmetrical, that is R_1 and R_2 are the same as each other, R_3 and R_4 are the same as each other, R_5 and R_6 are the same as each other, R_7 and R_8 are the same as each other.

In a preferred embodiment A1 is lysine, serine or threonine, preferably lysine. Preferably A3 and A4 are lysine, ornithine, histidine or arginine.

In a further preferred embodiment the hydrocarboxyl group is selected from:

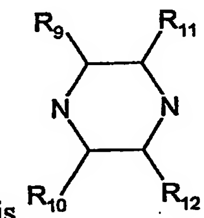
$-C(O)(CH_2)_{10}CH_3$
$-C(O)(CH_2)_{12}CH_3$
$-C(O)(CH_2)_{14}CH_3$
$-C(O)(CH_2)_{16}CH_3$
$-C(O)(CH_2)_{18}CH_3$
$-C(O)(CH_2)_{20}CH_3$
$-C(O)(CH_2)_7CH{=}CH(CH_2)_5CH_3$ natural mixture
$-C(O)(CH_2)_7CH{=}CH(CH_2)_7CH_3$ natural mixture
$-C(O)(CH_2)_7CH{=}CH(CH_2)_5CH_3$ Cis
$-C(O)(CH_2)_7CH{=}CH(CH_2)_7CH_3$ Cis
$-C(O)(CH_2)_7CH{=}CH(CH_2)_5CH_3$ Trans
$-C(O)(CH_2)_7CH{=}CH(CH_2)_7CH_3$ Trans
$-C(O)(CH_2)_7CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3$
$-C(O)(CH_2)_7(CH{=}CHCH_2)_3CH_3$
$-C(O)(CH_2)_3CH{=}CH(CH_2CH{=}CH)_3(CH_2)_4CH_3$
$-C(O)(CH_2)_7CHCH(CH_2)_7CH_3$
$-C(O)CHCHOH(CH_2)_2CH_3$ or

$-C(O)(CH_2)_{22}CH_3$.

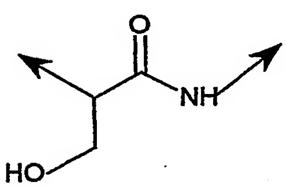
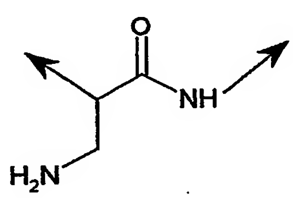
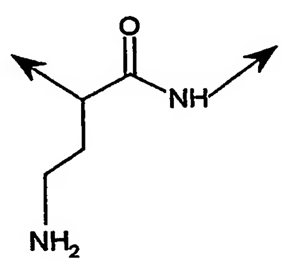
Most preferably the hydrocarboxyl group is selected from $(CH_2)_7$ $CH{=}CH(CH_2)_7CH_3$ natural mixture, $(CH_2)_7$ $CH{=}CH(CH_2)_7CH_3$ Cis and $(CH_2)_7$ $CH{=}CH(CH_2)_7CH_3$ Trans.

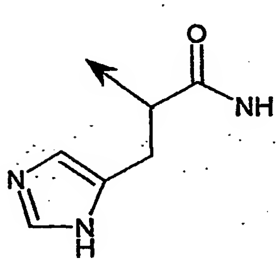
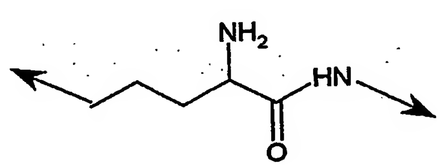
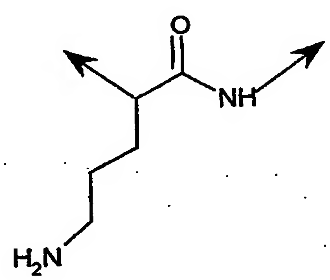
In a preferred embodiment m is 0, n is 2 to 4, X is (CH_2) or $(CH_2)_2$, Y is a bond and p is 0 to 4.

In a further preferred embodiment m is 0, n is 2 to 4, X is $NH(CH_2)qNH$, where q is 2 to 5, Y is a bond and p is 2 to 5.

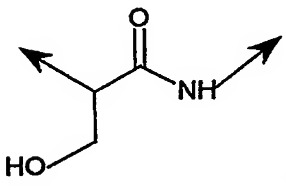
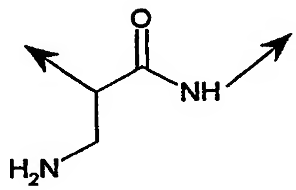
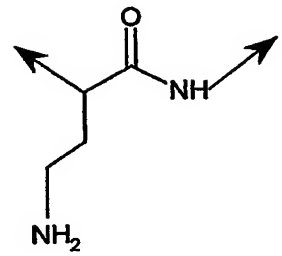
In another preferred embodiment m is 0, n is 2 to 4, X is  , where R_9, R_{10}, R_{11} and R_{12} are all H, Y is a bond and p is 2 to 5.

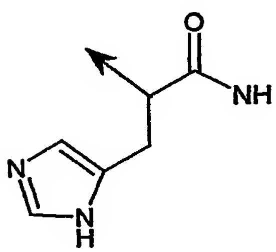
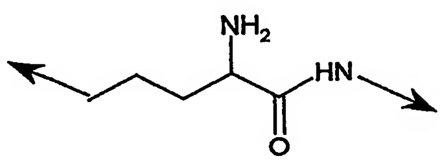
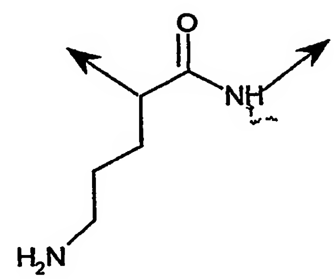
In a still further preferred embodiment m is 0, n is 2 to 4, X is (CH_2) or $(CH_2)_2$, p is 0 to 4 and Y is

 ,  ,  ,

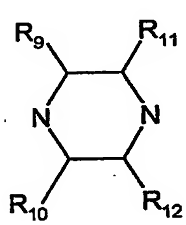
 ,  , or 

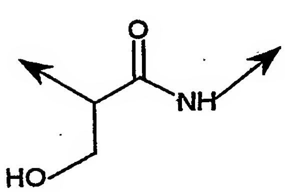
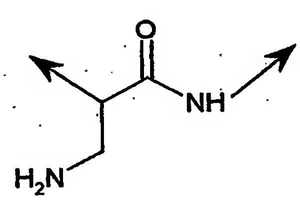
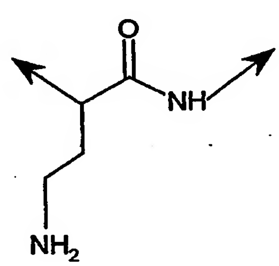
In a yet further preferred embodiment m is 0, n is 2 to 4, X is $NH(CH_2)qNH$, where q is 2 to 5, p is 2 to 5 and Y is

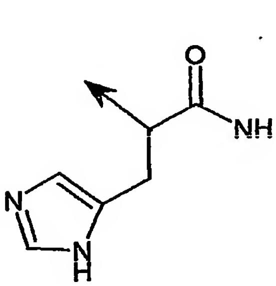
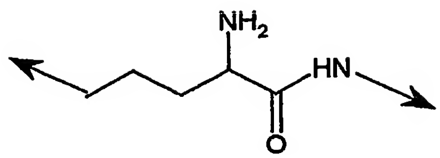
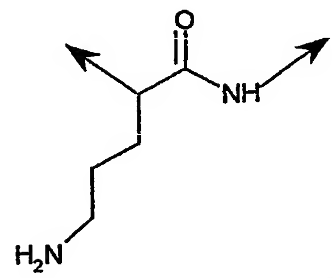
 ,  ,  ,

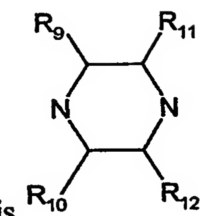
 ,  , or  .

In a yet still further preferred embodiment m is 0, n is 2 to 4, X is

 , where R_9, R_{10}, R_{11} and R_{12} are all H, p is 2 to 5 and Y is

 ,  ,  ,

,
, or


A further preferred embodiment is where X is , Y is a bond, p is 1 to 6 and n is 1 to 7.

Compounds of the present invention may be prepared from readily available starting materials using synthetic peptide chemistry well known to the skilled person. The scheme shown in Figure 1 shows a general scheme for the synthesis of the compounds of the invention wherein the hydrocarboxyl groups are linked to the α-amino group of a diaminoacid further linked to a polyamine backbone moiety by amide bonds, the scheme shown in Figure 2 shows a general scheme for the synthesis of the compounds of the invention wherein the hydrocarboxyl groups are linked to the terminal amino group of a diaminoacid further linked to a polyamine backbone moiety by amide bonds and the scheme shown in Figure 3 shows a general scheme for the synthesis of diaminoacid-aminoacid-polyamine:peptide based gemini compounds wherein an aminoacid is linked by an amide bond to the amino group (α or terminal) of a diaminoacid further linked to a polyamine moiety by an amide bond.

Another aspect of the invention relates to methods for using the diaminoacid-polyamine:peptide based gemini compounds. Such uses include facilitating the transfer of oligonucleotides and polynucleotides into cells for antisense, gene therapy and genetic immunisation (for the generation of antibodies) in whole organisms. Other uses include employing the compounds of the invention to facilitate

the transfection of polynucleotides into cells in culture when such transfer is required, in, for example, gene expression studies and antisense control experiments among others. Protocols for the preparation of such polynucleotides and antisense molecules are well known in the art (for example Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), Cohen, JS ed. Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1989)). The polynucleotides can be mixed with the compounds, added to the cells and incubated to allow polynucleotide uptake. After further incubation the cells can be assayed for the phenotypic trait afforded by the transfected DNA, or the levels of mRNA expressed from said DNA can be determined by Northern blotting or by using PCR-based quantitation methods for example the Taqman® method (Perkin Elmer, Connecticut, USA). Compounds of the invention offer a significant improvement, typically between 3 and 6 fold, in the efficiency of cellular uptake of DNA in cells in culture, compared with compounds in the previous art. In the transfection protocol, the gemini compound may be used in combination with one or more supplements to increase the efficiency of transfection. Such supplements may be selected from, for example:

(i) a neutral carrier, for example dioleyl phosphatidylethanolamine (DOPE) (Farhood, H., *et al* (1985) *Biochim. Biophys. Acta,* 1235-1289);

(ii) a complexing reagent, for example the commercially available PLUS reagent (Life Technologies Inc. Maryland, USA) or peptides, such as polylysine or polyornithine peptides or peptides comprising primarily, but not exclusively, basic amino acids such as lysine, ornithine and/or arginine. The list above is not intended to be exhaustive and other supplements that increase the efficiency of transfection are taken to fall within the scope of the invention.

In still another aspect, the invention relates to the transfer of genetic material in gene therapy using the compounds of the invention. For example the skilled person can develop gene delivery methodologies for use in gene therapy, involving the use of gemini surfactant compounds of the present invention, using protocols that are well known in the art. For example the use of surfactants for delivery of gene transfer vectors to the lung is reviewed in Weiss, DJ (2002) Molecular Therapy 6(2) pp148 to 152.

Yet another aspect of the invention relates to methods to effect the delivery of non-nucleotide based drug compounds into cells *in vitro* and *in vivo* using the compounds of the invention.

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"Amino acid" refers to dipolar ions (zwitterions) of the form $^{+}H_3NCH(R)CO_2^{-}$. They are differentiated by the nature of the group R, and when R is different from hydrogen can also be asymmetric, forming D and L families. There are 20 naturally occurring amino acids where the R group can be, for example, non-polar (e.g. alanine, leucine, phenylalanine) or polar (e.g. glutamic acid, histidine, arginine and lysine). In the case of un-natural amino acids R can be any other group which is not found in the amino acids found in nature.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNA's or RNA's containing one or more modified bases and DNA's or RNA's with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications have been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

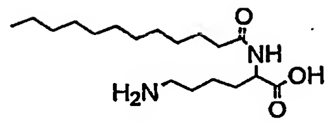
"Transfection" refers to the introduction of polynucleotides into cells in culture using methods involving the modification of the cell membrane either by chemical or physical means. Such methods are described in, for example, Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). The polynucleotides may be linear or circular, single-stranded or double-stranded and may include elements controlling replication of the polynucleotide or expression of homologous or heterologous genes which may comprise part of the polynucleotide.

The invention will now be described by way of the following examples.

EXAMPLES

Example 1:

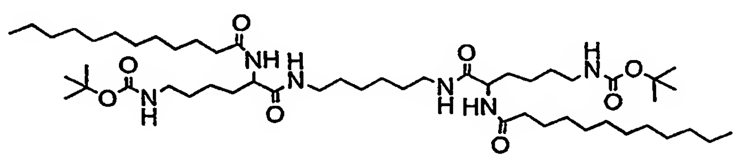
CR-110



To a solution of H-Lys(Boc)-OH (5.02 g, 20.4 mmol) and 20.5 mL of NaOH 1M in 85 Ml of water-acetone (1:2 v/v) cooled at 0°C was added dropwise 4.43 g (20.3 mmol) of dodecyl chloride and NaOH aq. 1M alternatively to maintain the pH over 9. After addition keep 10 minutes more stirring at 0°C. HCl 10% was added until pH 2. Filter the solid and wash with water until pH 7. Dry over P_2O_5. The solid is chromatographied on silica with $CHCl_3$- MeOH to yield 46% of compound CR-110 as a white solid. α_D^{20} -1.0 (c 1.48 , MeOH) ; IR(KBr)ν_{max} 3347, 2921, 2851, 1717, 1681, 1521 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) 4.26 (dd, 1H, J= 4.77, 8.92 Hz, C<u>H</u>-COOH), 2.94 (t, 2H, J= 6.7 Hz, CH_2N), 2.16 (t, 2H, J= 7.4 Hz, CH_2CON), 1.78-1.74 (m, 1H, HC<u>H</u>-CH(COOH), 1.63-1.45 (m, 7H, HC<u>H</u>-CH(COOH), <u>CH₂CH₂</u>N and <u>CH₂</u>CH₂CON), 1.35 (s, 9H, (C<u>H₃</u>)₃C), 1.22 (s, 16H, $CH_3(CH_2)_8$), 0.82 (t, 3H, J=6.8 Hz, CH_3); ^{13}C (75 MHz, CD_3OD) 176.32 <u>C</u>(O)NCH₂), 175.45 (COOH), 158.42 (C(O)NO), 79.73 (<u>C</u>(CH₃)₃), 54.84 (CH), 41.16 (CH_2N), 36.97, 33.04, 32.64, 30.74-29.99 (CH_2), 28.83 (CH_3), 26.98, 24.26, 23.71 (CH_2), 14.48 (CH_3).

Example 2:

CR-116

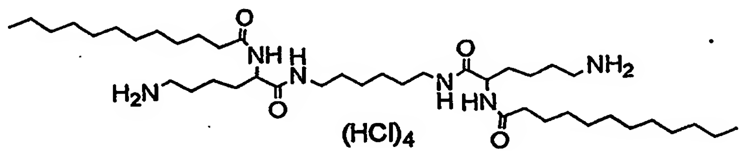


To a solution of 2.4 g (5.6 mmol) of CR-110 in THF at –20°C were added Et_3N (0.78 mL, 5.6 mmol) and EtOCOCl (0.55 mL, 5.6 mmol). The reaction was stirring at this temperature for 30 minutes and 246 mg(2.8 mmol) of 1,4-diaminobutane were added, after 1 hour more stirring at –20°C the reaction mixture was allowed to warm at room temperature and stirred overnight. Remove the solvent in vacuum, the residue was dissolved in $CHCl_3$ and washed with $NaHCO_3$ aq. saturated and brine and dried over MgSO4 anh. The obtained residue was chromatographied to give compound **CR-116** (50%) as a white solid ; α_D^{20} -10.06 (c 1.51 , MeOH); IR(KBr)ν_{max} 3415-3307, 2920, 2851, 1688, 1637, 1515 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) 4.17 (dd, 1H, J= 5.5, 8.5 Hz, C<u>H</u>-COOH), 3.12 (m, 2H, CH_2N), 2.96 (q, 2H, J= 6.4 Hz, CH_2N), 2.17 (t, 2H, J= 7.4 Hz, $CH_2C(O)N$), 1.69-1.64 (m, 1H, HC<u>H</u>-HC(COOH), 1.58-1.42 (m, 5H, HC<u>H</u>-HC(COOH), <u>CH₂</u>CH₂CO, <u>CH₂</u>CH₂N), 1.36 (s, 9H,

$(CH_3)_3C)$, 1.22 (s, 16H, $CH_3(CH_2)_8CH_2$), 0.88 (t, 2H, J=6.8 Hz, CH_3); ^{13}C (75 MHz, CD_3OD) 176.26, 174.46 C(O)NCH_2), 158.42 (OC(O)N), 79.93 ($\underline{C}(CH_3)_3$), 54.82 (CH), 41.11 and 39.96 (CH_2N), 36.89, 33.09, 32.92, 30.77-30.38 (CH_2), 28.85 (CH_3), 27.63, 26.94, 24.28, 23.74(CH_2), 14.48 (CH_3).

Example 3:

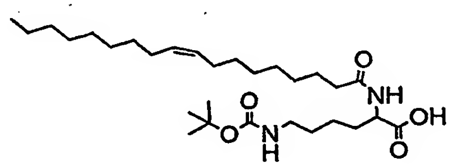
CR-117: GSN11



1.2299 g (1.35 mmol) of CR-116 were treated with EtOAc 4 M for 45 minutes. The solid was filtered and recrystalized from MeOH and EtOAc added to obtain the compound CR-117 as a white solid (49%): α_D^{20} -13.98 (c 1.76 , MeOH); IR(KBr)ν_{max} 3422, 3298, 3089, 2920, 2851, 1638 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) 4.20 (dd, 1H, J= 5.6, 8.4 Hz, C$\underline{H}$-COOH), 3.12 (m, 2H, $C\underline{H}_2N$), 2.84 (t, 2H, J= 6.4 Hz, CH_2N), 2.18 (t, 2H, J= 7.6 Hz, $CH_2C(O)N$), 1.74-1.72 (m, 1H, HC$\underline{H}$-CH(COOH), 1.69-1.34 (m, 5H, HC$\underline{H}$-CH(COOH) + $C\underline{H}_2CH_2CO$+ $C\underline{H}_2CH_2N$), 1.22 (s, 16H, $CH_3(CH_2)_8CH_2$), 0.82 (t, 2H, J=6.8 Hz, CH_3); ^{13}C (75 MHz, CD_3OD) 176.39, 174.22 $\underline{C}(O)NCH_2$), 54.59 (CH), 40.55, 39.99 (CH_2N), 33.08, 32.57, 30.76-30.41(CH_2), 28.23, 27.61, 26.93 (CH_2), 14.44 (CH_3); $C_{40}H_{80}Cl_2N_6O_4 \cdot H_2O$ 778.56 calc C 60.94 %,H 10.36 %, N 10.65 % found C 60.88%, H10.22%, N 10.08%

Example 4

RG 00/781

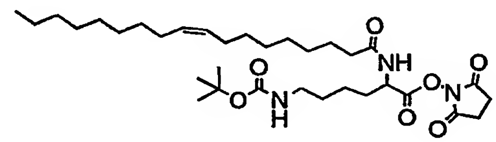


To a solution of *N*-ε-(*tert*butoxycarbonyl)-L-lysine (1.24 g, 5.03 mmol) in THF (140 mL) were added successively a solution of K_2CO_3 (0.75 g, 5.43 mmol, 1.08 eq.) in water (20 mL) and oleoyl succinimidate (1.92 g, 5.06 mmol, 1 eq.). The reaction was stirred at RT for 20 h and most of THF was evaporated. Water and $CHCl_3$ (30 mL each) were added and the organic layer was separated. The aqueous layer was acidified to pH 2 and extracted twice with $CHCl_3$ (2 x 30 mL). The organic layer

was washed with water and brine (20 mL each), dried (Na_2SO_4), filtered and evaporated to give an oil. Yield : 2.46 g (4.82 mmol, 96 %). ^{1}H NMR (400 MHz, d_6-DMSO): δ 12.4 (m, 1 H^{OH}), 7.92 (d, 1 H, $J = 7.8$, HN^{α}), 6.70 (t, 1 H, $J = 6.0$, HN^{ε}), 5.29 (m, 2 $CH^{9,10}$), 4.10 (dt, 1 H, J = 5.0, 8.9, CH^{α}), 2.85 (q, 2 H, $J = 6.2$, CH_2^{ε}), 2.07 (dt, 2 H, $J = 2.2$, 7.0, CH_2^{2}), 1.95 (q, 4 H, J = 6.0, $CH_2^{8,11}$), 1.62 (m, 1 H, CH^{β}), 1.51 (m, 1 H, CH^{β}), 1.45 (m, 2 H, CH_2^{3}), 1.33 (s, 9 H, $C(CH_3)_3$), 1.2 (m, 26 H, 2 $CH_2^{\gamma,\delta}$ and 10 CH_2 oleoyl), 0.82 (t, $J = 6.4$, 3 H, CH_3^{18}).

Example 5

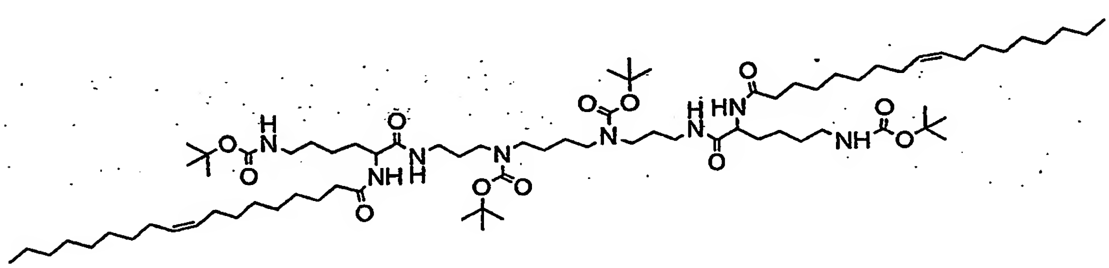
RG 00/366



To a solution of *N*-α-oleoyl-*N*-ε-(*tert*-butyloxycarbonyl)-L-Lysine (1.80 g, 3.52 mmol) in THF (80 mL) were added successively *N*-hydroxysuccinimide (0.41 g, 3.56 mmol, 1.01 eq.) and DCC (0.73 g, 3.54 mmol, 1.01 eq.). The reaction was stirred for 16 h at RT. The precipitate was filtered and washed with EtOAc (30 mL). The filtrate was concentrated and redissolved in EtOAc and filtered again. The residue was dissolved in $CHCl_3$ and precipitated with Et_2O to give N-α-oleate-N-ε-(*tert*-butyloxycarbonyl)-L-Lysinyl succinimidate as a white solid. Yield : 1.98 g (93 %). NMR ^{1}H (400 MHz, $CDCl_3$) : δ 6.11 (m, 1 H, HN^{α}), 5.38 (m, 2 H, $H^{9,10}$), 4.94 (m, 1 H, CH^{α}), 4.65 (m, 1 H, HN^{ε}), 3.12 (m, 2 H, CH_2^{ε}), 2.79 (s, 4 H, 2 CH_2^{Su}), 2.20 (t, $J = 6.1$, 2 H, CH_2^{2}), 2.00 (m, 5 H, CH^{β} and 2 $CH_2^{8,11}$), 1.84 (m, 1 H, CH^{β}), 1.63 (m, 2 H, CH_2^{3}), 1.48 (m, 4 H, 2 $CH_2^{\gamma,\delta}$), 1.37 (s, 9 H, 3 CH_3), 1.27 (m, 20 H, 10 CH_2 oleoyl), 0.83 (t, $J = 6.3$ Hz, 3 H, CH_3^{18}).

Example 6

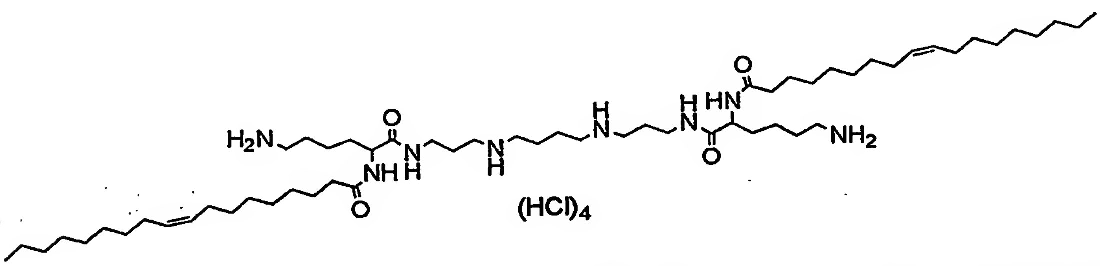
RG 00/250



To a solution of N^4,N^9-bis-(*tert*-butyloxycarbonyl)-1,12-diamino-4,9-diazadodecane (629 mg, 1.0 mmol) in THF (80 mL) and K_2CO_3 (0.29 g, 2.1 mmol, 2.1 eq.) in water (10 mL) was added a solution of *N*-α-oleoyl-*N*-ε-(*tert*-butyloxycarbonyl)-L-lysinyl succinimidate (1246 mg, 2.05 mmol, 2.05 eq.). The reaction was stirred overnight at RT. Most of the THF was evaporated and water (30 mL) was added. The aqueous layer was extracted with $CHCl_3$ (2 x 50 mL),. The organic layer was washed with water, 0.1 M HCl, water and brine (20 mL each), dried (Na_2SO_4), filtered, evaporated and purified by column chromatography on SiO_2 ($CHCl_3$ / MeOH : 95/5, Rf = 0.30) to give an oil. Yield : 1060 mg (0.76 mmol, 76 %). ^{1}H NMR (400 MHz, $CDCl_3$) : δ 7.30 (bs, 2 H, 2 $NHC^{1'}$), 6.33 (bs, 2 H, 2 NH^{α}), 5.31 (m, 4 H, 2 $CH^{9,10}$), 4.71 (bs, 2 H, 2 NH^{ε}), 4.41 (m, 2 H, 2 CH^{α}), 3.18 (m, 12 H, 2 $CH_2^{1'}$, 2 $CH_2^{3'}$), 3.08 (m, 4 H, 2 $CH_2^{3'}$ and 2 CH_2^{ε}), 2.18 (t, 4 H, J = 6.8, 2 CH_2^{2}), 1.98 (m, 8 H, 2 $CH_2^{8,11}$), 1.90 (m, 2 H, 2 CH^{β}), 1.79 (m, 2 H, 2 CH^{β}), 1.60 (m, 10 H, 2 $CH_2^{2'}$, 2 CH_2^{γ} and 2 CH_2^{3}), 1.45 (m, 26 H, 2 CH_2^{δ}, 2 $CH_2^{5'}$ and 2 $C(CH_3)_3$), 1.40 (s, 18 H, 2 $C(CH_3)_3$), 1.25 (m, 40 H, 2 x 10 CH_2^{Tail}), 0.86 (m, 6 H, J = 6.6, 2 CH_3^{18}). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 171.1, 174.3, 155.6, 155.7, 129.5, 129.3, 79.4, 78.5, 76.8, 52.4, 48.6, 46.4, 39.6, 36.1, 33.5, 31.4, 29.3, 29.2, 29.0, 28.8, 28.7, 28.0, 26.7, 25.3, 24.5, 22.2, 22.1, 13.7.

Example 7

RG 00/267: GSC 102

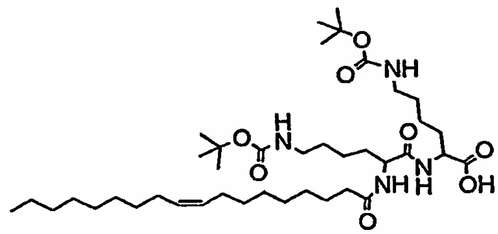


To a solution of RG 00/250 (1.04 g, 0.75 mmol) in MOH (20 mL) was added concentrated HCl (10 mL) and the reaction was stirred at RT for 2 h. The solvent were then removed and the residue redissolved in water (80 mL), filtered on a frit and evaporated again. The residue was redissolved in a minimum volume of methanol and precipitated with Et_2O to give, after filtration, a pale yellow solid. Yield : 0.734 g (0.65 mmol, 86 %). ^{1}H NMR (400 MHz, d_6-DMSO) : δ 9.02 (m, 4 H, 2 NH), 8.16 (t, 2 H, J = 6.0, 2 $NHC^{1'}$), 7.98 (s, 6 H, 2 $N^{\alpha}H$ and 2 $N^{\varepsilon}H_2$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.10 (q, 2 H, J = 7, 2 CH^{α}), 3.10 (hp, 4 H, J = 6.4, 2 $CH_2^{1'}$), 2.85 (m, 8 H, 2 $CH_2^{3'}$ and 2 $CH_2^{4'}$), 2.71 (m, 4 H, 2 CH_2^{ε}),

2.10 (AB, 4 H, $J = 6.4$, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,11}$), 1.76 (m, 4 H, 2 $CH_2^{2'}$), 1.68 (m, 4 H, 2 $CH_2^{5'}$), 1.65 – 1.42 (m, 12 H, 2 CH_2^{β}, 2 CH_2^{δ} and 2 CH_2^{3}), 1.25 (m, 44 H, 10 CH_2^{Ol} and 2 CH_2^{γ}), 0.83 (t, 6 H, 2 CH_3^{18}). MS (+ES) : 999.8 [M+Na].

Example 8

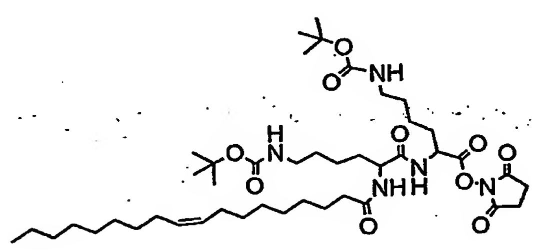
RG00/371



To a solution of *N*-α-oleoyl-*N*-ε-(*tert*-butyloxycarbonyl)-L-lysine (900 mg, 1.48 mmol) in THF (60 mL) were added successively a solution of potassium carbonate (225 mg, 1.63 mmol, 1.1 eq.) in water (6 mL) and *N*-ε-(tert-butyloxycarbonyl)-L-lysine (365 mg, 1.49 mmol, 1 eq.). The solution was then stirred for 16 h at RT. Most of THF was evaporated and pH of the aqueous solution was adjust to 2 and extract with $CHCl_3$ (2 x 80 mL). The organic layer was washed with water (50 mL) and brine (40 mL), dried (Na_2SO_4), filtered and evaporated. The oil obtained was then dissolved in a small quantity of $CHCl_3$ and Et_2O was added. The white solid was then collected. Yield : 1008 mg (1.46 mmol, 99 %). ^{1}H NMR (400 MHz, $CDCl_3$) : δ 12.60 (m, 1 H, COOH), 8.55 (m, 1 H, NH), 7.10 (m, 1 H, 1 NH), 6.70 (m, 1 H, 1 NH), 5.32 (m, 2 H, $CH^{9,10}$), 4.80 (m, 1 H, NH), 4.51 (m, 2 H, 2 CH^{α}), 3.08 (m, 4 H, 2 CH_2^{ε}), 2.20 (t, 2 H, $J = 7.0$, 2 CH_2^{2}), 1.99 (m, 4 H, $CH_2^{8,11}$), 1.60 (m, 4 H, 2 CH_2^{β}), 1.50 – 1.20 (m, 44 H), 0.87 (t, 3 H, $J = 6.8$, CH_3^{18}).

Example 9

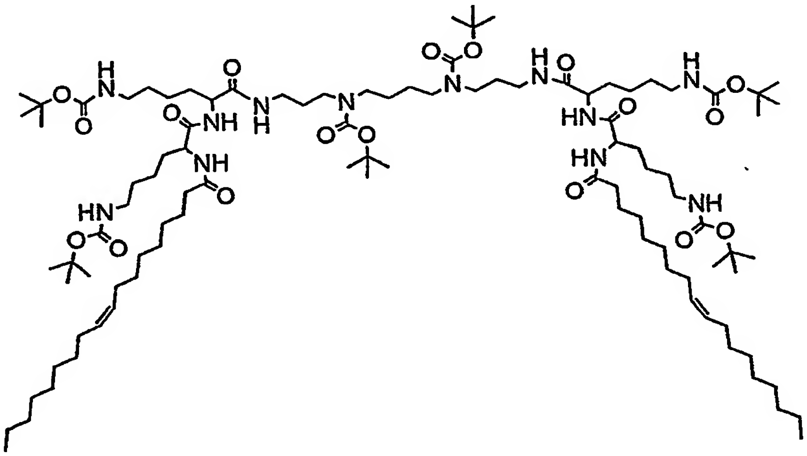
RG 00/376



To a solution of *N*–α-(*N*-α-Oleoyl-*N*-ε-(tert-butyloxycarbonyl)-L-lysyl)-*N*-ε-(tert-butyloxycarbonyl))-L-lysine (1008 mg, 1.46 mmol) in THF (40 mL) was added N-hydroxysuccinimide (177 mg, 1.49 mmol, 1.02 eq.) and DCC (311 mg, 1.50 mmol, 1.03 eq.). The reaction was stirred overnight at RT and the DCU was then filtered and washed with EtOAc. The solvent was then removed and the residue redissolved in EtOAc, the DCU filtered again and after evaporation a white solid was isolated. Yield : 1147 mg (1.36 mmol, 93 %).

Example 10

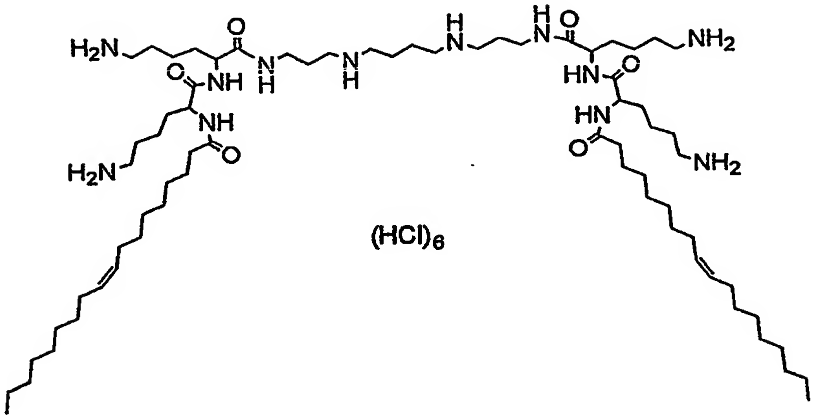
RG 00/384



To a solution of N^4,N^9-bis-(*tert*-butyloxycarbonyl)-1,12-diamino-4,9-diazadodecane (241 mg, 0.36 mmol) in THF (60 mL) and K_2CO_3 (0.10 g, 0.73 mmol, 2.1 eq.) in water (8 mL) was added a solution of *N*–α-(*N*-α-oleoyl-*N*-ε-(*tert*-butyloxycarbonyl)-L-Lysyl)-*N*-ε-(tert-butyloxycarbonyl))-L-lysyl succinimidate (600 mg, 0.72 mmol, 2.0 eq.) in THF (10 mL). The reaction was stirred overnight at RT. Most of the THF was evaporated and water (30 mL) was added. The aqueous layer was extracted with $CHCl_3$ (2 x 60 mL),. The organic layer was washed with water, 0.1 M HCl, water and brine (20 mL each), dried (Na_2SO_4), filtered, evaporated and purified on SiO_2 ($CHCl_3$ / MeOH : 9/1, Rf = 0.27) to give a white solid. Yield : 497 mg (0.27 mmol, 75 %). ^{1}H NMR (400 MHz, $CDCl_3$) : δ 8.40 (m, 2 H, 2 NH), 6.90 (m, 2 H, 2 NH), 6.40 (m, 2 H, 2 NH), 5.33 (m, 4 H, 2 $CH^{9,10}$), 4.85 (m, 4 H, 4 $N^{\varepsilon}H$), 4.40 (m, 4 H, 2 x 2 CH^{α}), 3.28 – 3.02 (m, 20 H, 2 x 2 CH_2^{ε}, 2 $CH_2^{1'}$, 2 $CH_2^{3'}$ and 2 $CH_2^{4'}$), 2.22 (m, 4 H, 2 CH_2^{2}), 1.99 (m, 8 H, 2 $CH_2^{8,11}$), 1.80 (m, 4 H, 2 CH_2^{β}), 1.72 – 1.25 (m, 126 H), 0.83 (t, 6 H, J = 6.8, 2 CH_3^{18}).

Example 11

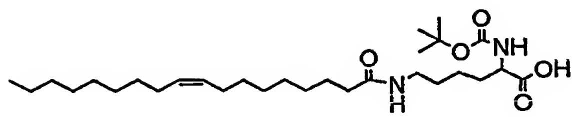
RG 00/404



To a solution of RG 00/384 (470 mg, 0.255 mmol) in MeOH (10 mL) was added concentrated HCl (10 mL) and the reaction was stirred at RT for 1 h. The solvents were removed under vacuum and the residue redissolved into water (80 mL), filtered and evaporated again. The residual oil was dissolved in MeOH and precipitated with Et_2O to give a yellow powder. Yield : 284 mg (0.194 mmol, 76 %). ^1H NMR (400 MHz, d_6-DMSO) : δ 9.10 (m, 4 H, 2 NH_2^+), 8.18 (m, 4 H, *4* NHC), 8.10 – 7.98 (m, 16 H, 2 x 2 $N^{\alpha}H$ and 2 x 2 $N^{\varepsilon}H_3^+$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.18 (m, 2 H, CH^{α}), 4.11 (m, 2 H, 2 CH^{α}), 3.10 (m, 4 H, 2 $CH_2^{1'}$), 2.85 (m, 8 H, 2 $CH_2^{3'}$ and 2 $CH_2^{4'}$), 2.71 (m, 8 H, 2 x 2 CH_2^{ε}), 2.10 (m, 4 H, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,11}$), 1.80 – 1.39 (m, 28 H), 1.25 (m, 48 H, 10 CH_2^{Ol} and 2 x 2 CH_2^{γ}), 0.83 (t, 6 H, J = 6.8, 2 CH_3^{18}).

Example 12

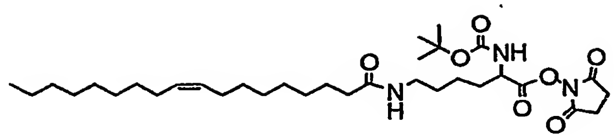
RG 00/278



To a solution of *N*-α-(tert-butyloxycarbonyl)-L-Lysine (779 mg, 3.16 mmol) in THF (80 mL) were added successively a solution of potassium carbonate (0.524 g, 3.79 mmol, 1.2 eq.) in water (10 mL) and oleoyl succinimidate (1.20 g, 3.16 mmol, 1 eq.). The reaction is stirred overnight at room temperature. Most of THF was evaporated and water (40 mL) was added. The aqueous layer was acidified to pH 2 and extracted with $CHCl_3$ (3 x 60 mL). The combined organic layers were washed with water (30 mL) and brine (40 mL), dried over sodium sulphate, filtered and evaporated to give N-α-(tert-butyloxycarbonyl)-N-ε-oleoyl-L-lysine as a colourless oil. Yield : 1.31 g (2.56 mmol, 81 %). ^1H NMR (400 MHz, $CDCl_3$) : d 5.78 (t, 1 H, J = 8.0, NH^{ε}), 5.33 (m, 2 H, $CH^{9,10}$), 5.27 (d, 1 H, J = 7.8, NH^{α}), 4.27 (m, 1 H, CH^{α}), 3.24 (q, 2 H, J = 8.0, CH_2^{ε}), 2.26 (t, 2 H, J = 6.8, CH_2^{2}), 1.98 (m, 4 H, $CH_2^{8,11}$), 1.85 (m, 1 H, CH^{β}), 1.70 (m, 1 H, CH^{β}), 1.60 (m, 2 H, CH_2^{3}), 1.55 (m, 2 H, CH_2^{δ}), 1.43 (s, 9 H, $C(CH_3)_3$), 1.40 (m, 2 H, CH_2^{γ}), 1.27 (m, 20 H, 10 CH_2^{Tail}), 0.87 (m, 3 H, J = 6.6, CH_3^{18}). HRMS (+ES) : 533.40327 calculated for $C_{29}H_{54}O_5N_2Na$ found 533.39110.

Example 13

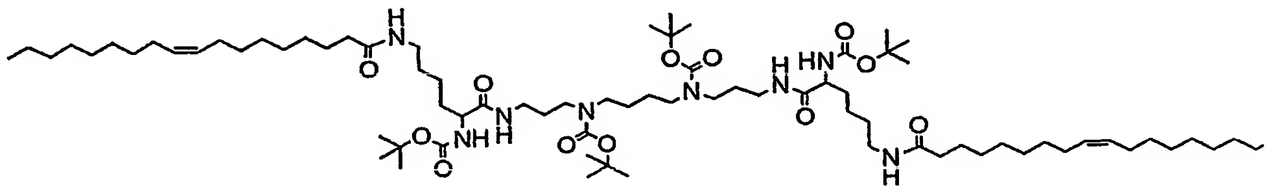
RG 00/281



To a solution of *N*-α-(tert-butyloxycarbonyl)-*N*-ε-oleoyl-L-Lysine (1.80 g, 3.52 mmol) in THF (80 mL) were added successively N-hydroxysuccinimide (0.41 g, 3.56 mmol, 1.01 eq.) and DCC (0.73 g, 3.54 mmol, 1.01 eq.). The reaction was stirred for 16 h at RT. The precipitate was filtered and washed with EtOAc (30 mL). The filtrate was concentrated and redissolved in EtOAc and filtered again. The residue was dissolved in $CHCl_3$ and precipitated with Et_2O to give *N*-α-oleate-*N*-ε-(tert-butyloxycarbonyl)-L-Lysinyl succinimidate as a white solid. Yield : 1.98 g (93 %). ^{1}H NMR (400 MHz, $CDCl_3$) : d 5.80 (t, 1 H, J = 8.0, NH^{ε}), 5.32 (m, 2 H, $CH^{9,10}$), 5.12 (d, 1 H, J = 7.8, NH^{α}), 4.66 (m, 1 H, CH^{α}), 3.24 (q, 2 H, J = 8.0, CH_2^{ε}), 2.82 (s, 4 H, 2 CH_2^{Su}), 2.14 (t, 2 H, J = 6.8, CH_2^{2}), 1.98 (m, 4 H, $CH_2^{8,11}$), 1.90 (m, 2 H, 2 CH^{β}), 1.60 (m, 2 H, CH_2^{3}), 1.55 (m, 2 H, CH_2^{δ}), 1.44 (s, 9 H, $C(CH_3)_3$), 1.39 (m, 2 H, CH_2^{γ}), 1.25 (m, 20 H, 10 CH_2^{Tail}), 0.86 (m, 3 H, J = 6.6, CH_3^{18}).

Example 14

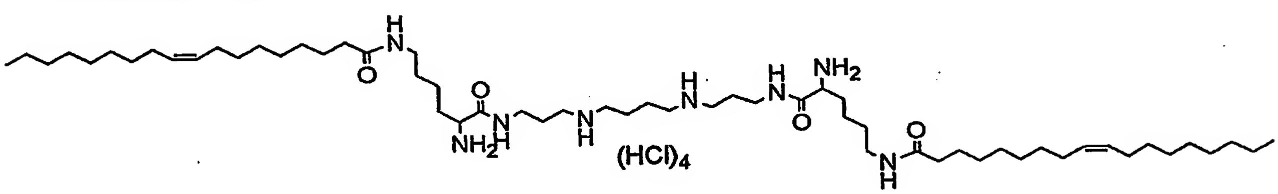
RG 00/286



To a solution of N^4,N^9-bis-(tert-butyloxycarbonyl)-1,12-diamino-4,9-diazadodecane (414 mg, 0.659 mmol) in THF (60 mL) and K_2CO_3 (200 mg, 1.2 mmol, 2.2 eq.) in water (7 mL) was added a solution of *N*-α-(tert-butyloxycarbonyl)-*N*-ε-oleoyl-L-lysinyl succinimidate (800 mg, 1.32 mmol, 2.0 eq.) in THF (35 mL). The reaction was stirred overnight at RT. Most of the THF was evaporated and water (30 mL) was added. The aqueous layer was extracted with $CHCl_3$ (2 x 30 mL),. The organic layer was washed with water, 0.1 M HCl, water and brine (30 mL each), dried (Na_2SO_4), filtered, evaporated and purified on SiO_2 ($CHCl_3$ / MeOH : 95/5, Rf = 0.27) to give an oil. Yield : 740 mg (0.533 mmol, 81 %). ^{1}H NMR (400 MHz, $CDCl_3$) : δ 7.20 (bs, 2 H, 2 $NHC^{1'}$), 5.72 (bs, 2 H, NH^{ε}), 5.33 (m, 4 H, 2 $CH^{9,10}$), 5.25 (bs, 2 H, 2 NH^{α}), 4.08 (m, 2 H, 2 CH^{α}), 3.24 (m, 12 H, 2 $CH_2^{1'}$, 2 $CH_2^{4'}$ and 2 CH_2^{ε}), 3.12 (m, 4 H, 2 $CH_2^{3'}$), 2.13 (t, 4 H, J = 6.8, 2 CH_2^{2}), 1.98 (m, 8 H, 2 $CH_2^{8,11}$), 1.80 (m, 2 H, 2 CH^{β}), 1.60 (m, 10 H, 2 $CH_2^{2'}$, 2 CH^{β} and 2 CH_2^{3}), 1.50 (m, 4 H, 2 CH_2^{δ}), 1.47 (m, 4 H, 2 $CH_2^{5'}$), 1.45 (s, 18 H, 2 $C(CH_3)_3$), 1.42 (s, 18 H, 2 $C(CH_3)_3$), 1.37 (m, 4 H, 2 CH_2^{γ}), 1.25 (m, 40 H, 2 x 10 CH_2^{Tail}), 0.86 (m, 6 H, J = 6.6, 2 CH_3^{18}).

Example 15

RG 00/320 : GSC 101

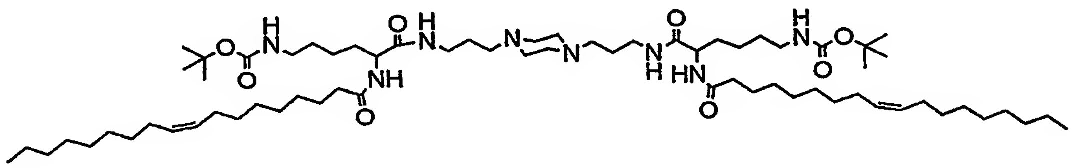


To a solution of RG 00/296 (750 mg, 0.540 mmol) in MeOH (10 mL) was added concentrated HCl (10 mL). The reaction was stirred at RT for 1 h and then evaporated. The residue was redissolved in water (60 mL) and filtered. Water was evaporated and the residue dissolved in a small amount of MeOH and precipitated with Et_2O to give a yellow solid. Yield : 533 mg (0.470 mmol, 90 %). ^{1}H NMR (400 MHz, d_6-DMSO) : δ 9.02 (m, 4 H, 2 NH_2^+), 8.83 (t, 2 H, J = 6.0, 2 $NHC^{1'}$), 8.30 (d, 6 H,

$J = 4.0$, 2 $N^{\alpha}H_3^+$), 8.83 (t, 2 H, $J = 6.0$, 2 $N^{\varepsilon}H$), 5.30 (m, 4 H, 2 $CH^{9,10}$), 3.70 (q, 2 H, $J = 7$, 2 CH^{α}), 3.22 (m, 2 H, 2 $CH^{1'}$), 3.13 (m, 2 H, 2 $CH^{1'}$), 2.97 (m, 4 H, 2 CH_2^{ε}), 2.71(m, 8 H, 2 $CH_2^{3'}$ and 2 $CH_2^{4'}$), 2.10 (t, 4 H, $J = 7.3$, 2 CH_2^{2}), 1.95 (q, 8 H, J = 6.0, 2 $CH_2^{8,11}$), 1.82 (h, 4 H, J = 7.0, 2 $CH_2^{2'}$), 1.68 (m, 8 H, 2 CH_2^{β} and 2 $CH_2^{5'}$), 1.43 (qu, 4 H, $J = 6.2$, 2 CH_2^{3}), 1.35 (m, 4 H, 2 CH_2^{δ}), 1.25 (m, 44 H, 2 x 10 CH_2^{Ol} and 2 CH_2^{γ}), 0.82 (t, 6 H, 2 CH_3^{18}). MS (+ES) : 999.8 [M+Na].

Example 16

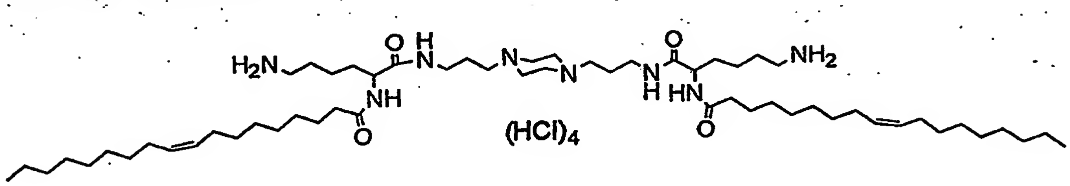
RG 00/518



To a solution of activated aminoacid RG00/366 (610 g, 1.0 mmol) in THF (45 mL) was added bis-*N*-aminopropyl-piperazine (0.081 mL, 0.5 mmol, 0.5 eq.) and then potassium carbonate (0.15 g, 1.1 mmol, 2.2 eq.) in water (10 mL) and the reaction was stirred at RT for 20 h. Most of the THF was removed under vacuum, $CHCl_3$ was added and the organic layer was extracted, washed with water (20 mL), dried (Na_2SO_4), filtered and evaporated. The residue was purified by column chromatography on silica ($CHCl_3$ / MeOH : 8.5 / 1.5, Rf = 0.3) to give a white solid. Yield : 490 mg (0.413 mmol, 83 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (m, 2 H, 2 $NHC^{1'}$), 6.46 (m, 2 H, 2 $N^{\alpha}H$), 5.32 (m, 4 H, 2 $CH^{9,10}$), 4.86 (m, 2 H, 2 N^{ε}Hboc), 4.33 (q, 2 H, $J =$, 2 CH^{α}), 3.38 (m, 2 H, $CH^{1'}$), 3.28 (m, 2 H, $CH^{1'}$), 3.05 (m, 4 H, 2 CH_2^{ε}), 2.47 (m, 12 H, 2 $CH_2^{3'}$ and 4 $CH_2^{2'}$), 2.18 (t, 4 H, $J =$, 2 CH_2^{2}), 1.99 (m, 8 H, 2 $CH_2^{8,10}$), 1.82 – 1.54 (m, 12 H, 2 $CH_2^{2'}$, 2 CH_2^{3} and 2 CH_2^{β}), 1.48 (m, 4 H, 2 CH_2^{γ}), 1.42 (s, 18 H, 2 $(CH_3)_3$), 1.21 (m, 24 H, 10 CH_2^{Ol} and 2 CH_2^{γ}), 0.87 (t, 6 H, $J = 6.4$, 2 CH_3^{18}). ^{13}C NMR (400 MHz, CD_3OD): δ 175.2, 173.4, 157.5, 129.9, 129.8, 78.8, 56.0, 53.8, 52.9, 41.3, 40.1, 37.7, 35.9, 32.1, 31.9, 29.9, 29.6, 29.5, 29.4, 29.3, 27.9, 27.2, 26.2, 26.0, 23.3, 22.8, 13.5.

Example 17

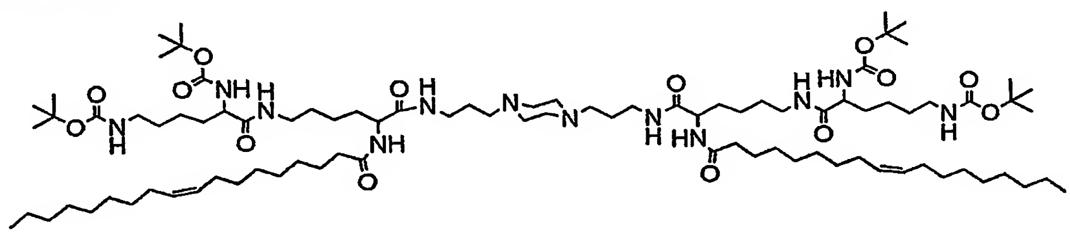
RG 00/522 = GSC 170



To a solution of protected RG00/518 (490 mg, 0.413 mmol) in MeOH (10 mL) was added concentrated HCl (10 mL). The reaction was stirred for 1 h and the solvent was then evaporated. The residue was redissolved in water (40 mL), filtered and evaporated. In this case it was impossible to precipitate the compound using MeOH / Et_2O. A white solid was collected. Yield : 381 mg (0.337 mmol, 81 %). HRMS (+ES) : 985.8879 calculated for $C_{58}H_{113}N_8O_4$, found 985.8890.
Note : a similar procedure using TFA and neutralisation with K_2CO_3 was used to isolate the free amine in a quantitative yield. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.78 (2 d, 4 H, J = 8.0, 4 NHCO), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.12 (q, 2 H, J = 6.2, 2 CH^{α}), 3.04 (m, 4 H, 2 $CH_2^{1'}$), 2.47 (m, 8 H, 4 CH_2^{4}), 2.29 (m, 4 H, 2 NH_2), 2.19 (t, 4 H, J = 6.2, 2 $CH_2^{3'}$), 2.05 (m, 4 H, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.35 – 1.69 (m, 12 H, 2 $CH_2^{2'}$, 2CH_2^{3} and 2 CH_2^{β}), 1.21 (m, 26 H, 10 CH_2^{Ol} and CH_2^{δ} and CH_2^{γ}), 0.82 (t, 6 H, J = 6.4, 2 CH_3^{18}).

Example 18

RG 00/794

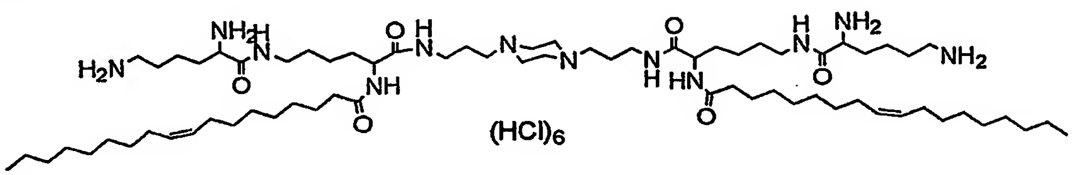


To a solution of bis aminocompound (150 mg, 0.152 mmol) in THF (40 mL) was added successively a solution of K_2CO_3 (42 mg, mmol, 2.1 eq.) in water (2 mL) and *N,N*-bis-(tertbutoxycarbonyl)-L-lysinyl succinimidate (140 mg, 0.304 mmol, 2.0 eq.) in THF (10 mL). The reaction was then stirred for 16 h at RT. Most of THF was evaporated and the residue redissolved in $CHCl_3$. Water (10 mL) was added and the organic layer extracted, washed with water (2 x 10 mL) and brine (20 mL). After drying (Na_2SO_4), filtration and evaporation, the residue is purified on SiO_2 (eluent : $CHCl_3$ / MeOH / NH_4OH: 87 / 12 / 1, Rf = 0.28). Et_2O is then added and the resulting white solid filtered off. Yield : 0.124 g (0.076 mmol, 50 %). ^{1}H NMR (400 MHz, d^6-DMSO) : δ 7.75 (m, 4 H, 2 $NH^{\alpha1}$ and 2 NHC^1), 7.68 (t, 2 H, J = , 2 $NH^{\varepsilon1}$), 6.69 (t, 2 H, J = , 2 $NH^{\varepsilon2}$), 6.63 (d, 2 H, J = , 2 $NH^{\alpha2}$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.10 (q, 2 H, J = , 2 $CH^{\alpha1}$), 3.78 (q, 2 H, J = , 2 $CH^{\alpha2}$), 3.00 (m, 6 H, 2 $CH_2^{\varepsilon1}$ and 2 $CH^{1'}$), 2.95 (m, 2 H, 2 $CH^{1'}$), 2.84 (m, 4 H, 2 $CH_2^{\varepsilon2}$), 2.29 (m, 8 H, 4 $CH_2^{4'}$), 2.19 (m, 4 H, 2 $CH_2^{3'}$), 2.06 (t,

4 H, $J=$, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.55 – 1.4 (m, 16 H), 1.32 (s, 36 H, 4 $C(CH_3)_3$), 1.20 (m, 48 H), 0.82 (t, 6 H, $J=6.4$, 2 CH_3^{18}).

Example 19

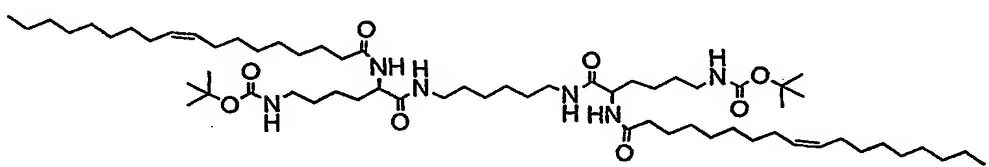
RG00/813 = GSC 184



To a solution of RG00/794 (124 mg, 0.0755 mmol) in MeOH (5 mL) was added concentrated HCl (5 mL). The reaction was stirred at RT for 1 h and the solvent were then removed under vacuum. The residue was dissolved in water, filtered and evaporated. The compound was dissolved in a minimum amount of MeOH and precipitated with Et_2O. The resulting solid was filtered and collected. Yield : 0.102 g (0.070 mmol, 93 %). ^{1}H NMR (400 MHz, d_6-DMSO) : δ 8.66 (d, 2 H, $J=7.8$, 2 $NH^{\varepsilon1}$), 8.28 (m, 6 H, 2 $N^{\alpha}H_3^+$), 8.09 (m, 2 H, 2 NHC^{1}), 8.05 (m, 6 H, 2 $N^{\varepsilon}H_3^+$), 7.98 (d, 2 H, $J=7.0$, 2 $N^{\alpha}H$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.09 (m, 2 H, 2 $CH^{\alpha1}$), 3.72 (m, 2 H, 2 $CH^{\alpha2}$), 3.65 (m, 2 H, 2 NH^+), 3.10 (m, 12 H, 2 $CH_2^{\varepsilon1}$, 2 $CH_2^{3'}$ and 2 $CH_2^{1'}$), 2.74 (m, 8 H, 2 $CH_2^{\varepsilon2}$), 2.11 (t, 4 H, $J=7.2$, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.82 (m, 2 H, 2 $CH_2^{\delta1}$), 1.70 (m, 2 H, 2 $CH_2^{\beta2}$), 1.57 (m, 6 H, 2 $CH_2^{\delta2}$ and 2 $CH^{\beta1}$), 1.50 – 1.15 (m, 66 H), 0.84 (t, 6 H, $J=6.4$, 2 CH_3^{18}). MS (+ES) : 1264.9 [M+Na].

Example 20

RG 00/787

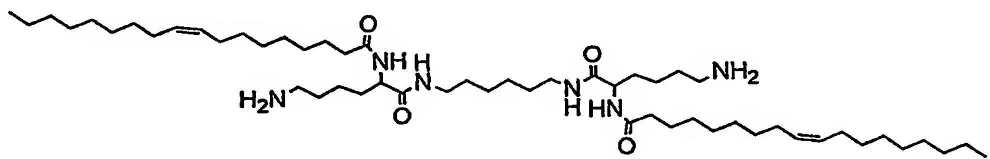


To a solution of 1,6-diaminohexane (72 mg, 0.62 mmol) in THF (60 mL) and K_2CO_3 (180 mg, 1.30 mmol, 2.1 eq.) in water (10 mL) was added a solution of *N*-α-oleoyl-*N*-ε-(*tert*-butyloxycarbonyl)-L-lysinyl succinimidate (750 mg, 1.23 mmol, 2 eq.). The reaction was stirred overnight at RT. Most of

the THF was evaporated and water (30 mL) was added. The aqueous layer was extracted with $CHCl_3$ (2 x 50 mL). The organic layer was washed with water, 0.1 M HCl, water and brine (20 mL each), dried (Na_2SO_4), filtered, evaporated and purified by column chromatography on SiO_2 ($CHCl_3$ / MeOH : 9/1, Rf = 0.33) to give an oil. Yield : 650 mg (0.59 mmol, 95 %). ^{1}H NMR (400 MHz, d_6-DMSO) : δ 7.73 (m, 4 H, 2 $N^{\alpha}H$ and 2 $N^{1'}H$), 6.68 (t, 2 H, J = 5.0, 2 $N^{\varepsilon}H$), 5.28 (m, 4 H, 2 $CH^{9,10}$), 4.12 (m, 2 H, 2 CH^{α}), 2.99 (q, 4 H, J = 6.4, 2 $CH^{1'}$), 2.83 (q, 4 H, J = 6.6, 2 CH_2^{ε}), 2.07 (dt, 4 H, J = 3.2, 7.0, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,11}$), 1.52 (m, 2 H, 2 CH^{β}), 1.42 (m, 6 H, 2 CH_2^{3} and 2 CH^{β}), 1.32 (s, 18 H, 2 $C(CH_3)_3$), 1.31 – 1.15 (m, 56 H, 2 x 10 CH_2^{Tail}, 2 CH_2^{δ}, 2 CH_2^{γ}, 2 $CH_2^{2'}$ and 2 $CH_2^{3'}$), 0.82 (t, 6 H, J = 6.8, 2 CH_3^{18}).

Example 21

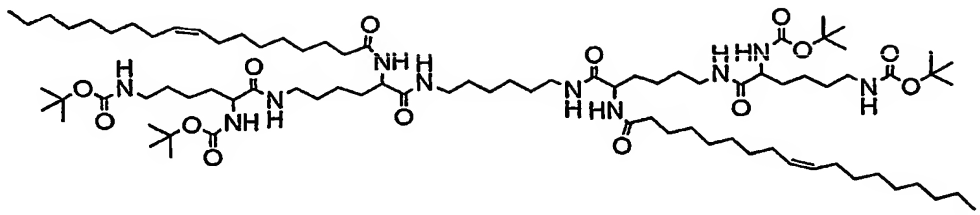
RG 00/873: GSN 14



To a solution of protected compound (640 mg, 0.581 mmol) in CH_2Cl_2 (10 mL) was added TFA (10 mL). The reaction was stirred at RT for 1 h and then evaporated (using several Et_2O (10 mL) to coevaporate). The oily residue was then dissolved in CH_2Cl_2, washed with 10 % aqueous K_2CO_3 (10 mL), water and brine. The organic phase was dried (Na_2SO_4), filtered and evaporated to give a pale brown solid which was triturated with Et_2O, filtered and dried to give a white solid. Yield : 460 mg (0.510 mmol, 88 %). The deprotection can be carried out using concentrated HCl in methanol giving the hydrochloric salt named GSN 14. ^{1}H NMR (400 MHz, d_6-DMSO) : δ 7.80 (m, 4 H, 2 $N^{\alpha}H$ and 2 $N^{1'}H$), 5.28 (m, 4 H, 2 $CH^{9,10}$), 4.16 (m, 2 H, 2 CH^{α}), 3.20 (bs, 4 H, 2 NH_2), 2.99 (q, 4 H, J = 6.4, 2 $CH^{1'}$), 2.53 (m, 4 H, 2 CH_2^{ε}), 2.10 (dt, 4 H, J = 3.2, 7.0, 2 CH_2^{2}), 1.91 (m, 8 H, 2 $CH_2^{8,11}$), 1.52 (m, 2 H, 2 CH^{β}), 1.48 (m, 2 H, 2 CH^{β}), 1.42 (m, 4 H, 2 CH_2^{3}), 1.31 – 1.15 (m, 56 H, 2 x 10 CH_2^{Tail}, 2 CH_2^{δ}, 2 CH_2^{γ}, 2 $CH_2^{2'}$ and 2 $CH_2^{3'}$), 0.81 (t, 6 H, J = 6.8, 2 CH_3^{18}).

Example 22

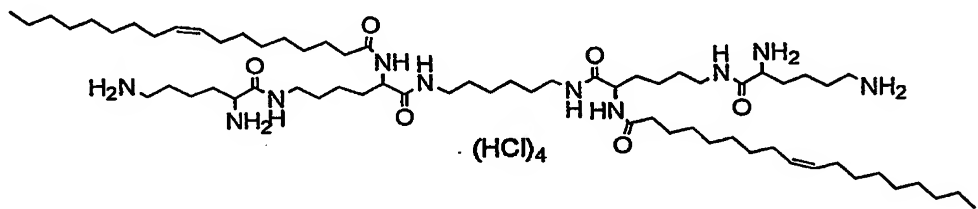
RG 00/874



To a 1/9 mixture of water and THF (20 mL) containing RG 00/873 (100 mg, 0.111 mmol) and potassium carbonate (32 mg, 0.232 mmol, 2.1 eq.) was added *N,N*-bis-(tert-butyloxycarbonyl)-L-lysinyl succinimidate (103 mg, 0.232 mmol, 2.1 eq.). The reaction was stirred for 20 h at RT. Most of THF was removed and the residue diluted with water (10 mL) and $CHCl_3$ (40 mL). The organic layer was decanted and washed successively with water (10 mL), 0.1 M HCl (20 mL), water (10 mL) and brine (25 mL). The organic layer was dried over sodium sulphate, filtered and evaporated. The resulting oil was crystallised from Et_2O. The white solid was collected. Yield : 164 mg (0.105 mmol, 95 %).

Example 23

RG 00/875 = GSC 197

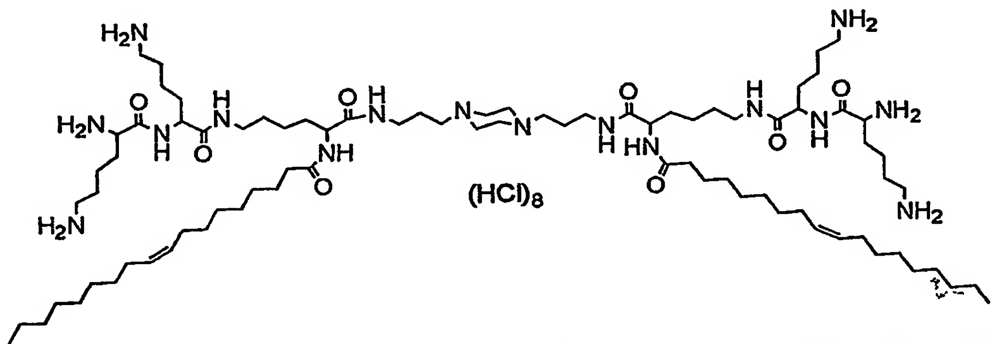


To a solution of RG 00/874 (160 mg, 0.103 mmol) in methanol (5 mL) is added concentrated HCl (5 mL). The reaction is stirred for 1 h and then evaporated to dryness. The residue is then dissolved in water (30 mL), filtered on sintered frit funnel (N° 3), evaporated to dryness using EtOH to coevaporate. The residue is dissolved in a small amount of methanol and precipitated with Et_2O to give the desired compound as a pale brown solid. Yield : 124 mg (0.951 mmol, 95 %). ^{1}H NMR (400 MHz, d_6-DMSO) : δ 8.59 (t, 2 H, J = 5.0, 2 $N^{1'}H$), 8.22 (m, 6 H, 2 $N^{\alpha 2}H_3^+$), 7.96 (m, 6 H, 2 $N^{\varepsilon 2}H_3^+$), 7.89 (d, 2 H, J = 8.0, 2 $N^{\alpha 1}H$), 7.89 (t, 2 H, J = 5.8, 2 $N^{\varepsilon 1}H$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.14 (dt, 2 H, J

= 5.4, 8.0, 2 $CH^{\alpha1}$), 3.70 (m, 2 H, 2 $CH^{\alpha2}$), 3.05 (m, 4 H, 2 $CH_2^{1'}$), 2.98 (q, 4 H, J = 5.8, 2 $CH_2^{\varepsilon1}$), 2.72 (m, 4 H, 2 $CH_2^{\varepsilon2}$), 2.09 (t, 4 H, J = 7.0, 2 CH_2^{2}), 1.94 (m, 8 H, 2 $CH_2^{8,11}$), 1.69 (m, 2 H, 2 $CH_2^{\beta2}$), 1.55 (m, 6 H, 2 $CH_2^{\delta2}$ and 2 $CH^{\beta1}$), 1.48 – 1.15 (m, 56 H), 0.81 (t, 6 H, J = 6.6, 2 CH_3^{18}).

Example 24

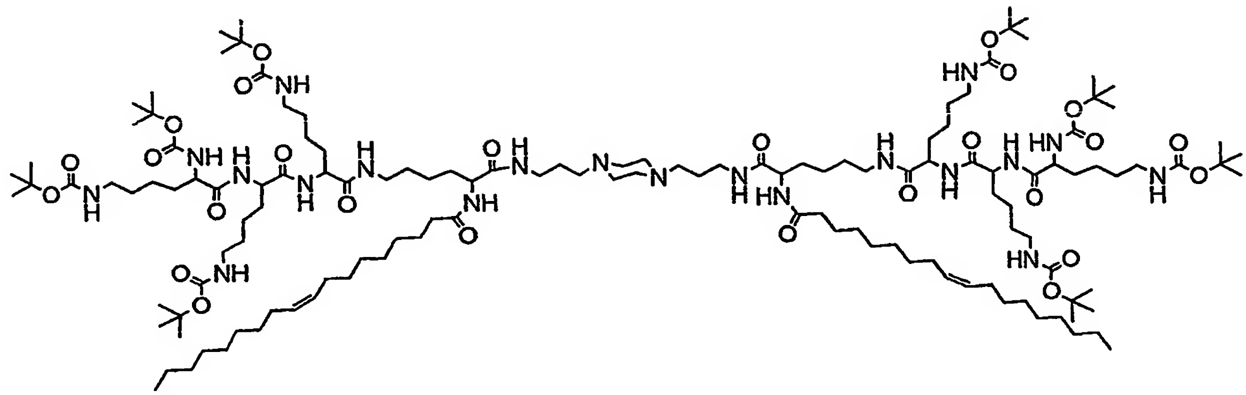
RG 00/804



To a solution of RG00/794 (110 mg, 0.052 mmol) in MeOH (7 mL) was added concentrated HCl (7 mL). The reaction was stirred at RT for 1 h and the solvent were then removed under vaccuum using EtOH to coevaporate. The residue was dissolved in water, filtered and evaporated. The compound was dissolved in a minimum amount of MeOH and precipitated with Et_2O. The resulting solid was filtered and collected as a white powder. Yield : 88 mg (0.049 mmol, 94 %). ^{1}H NMR (400 MHz, d_6-DMSO) : δ 8.69 (d, 2 H, J = 7.8, 2 $NH^{\alpha2}$), 8.30 (m, 2 H, 2 $NH^{\alpha1}$), 8.12 (m, 2 H, 2 $NHC^{1'}$), 7.95 (m, 12 H, 2 $NH^{\varepsilon2}$, 2 $NH^{\varepsilon3}$ and $NH^{\alpha3}$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.20 (m, 2 H, 2 $CH^{\alpha1}$), 4.08 (m, 2 H, 2 $CH^{\alpha3}$), 3.84 (m, 2 H, 2 $CH^{\alpha2}$), 3.65 (m, 2 H, 2 NH^{+}), 3.10 (m, 12 H, 2 $CH_2^{3'}$ and 2 $CH_2^{4'}$), 3.05 (m, 2 H, 2 $CH^{1'}$), 2.95 (m, 2 H, 2 $CH^{1'}$), 2.74 (m, 8 H, 2 $CH_2^{\varepsilon1}$ and 2 $CH_2^{\varepsilon2}$), 2.11 (t, 4 H, J = 7.2, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.80 – 1.12 (m, 82 H), 0.84 (t, 6 H, J = 6.4, 2 CH_3^{18}). MS (+ES) : m/z $[M+H]^{2+}$ 750.1.

Example 25

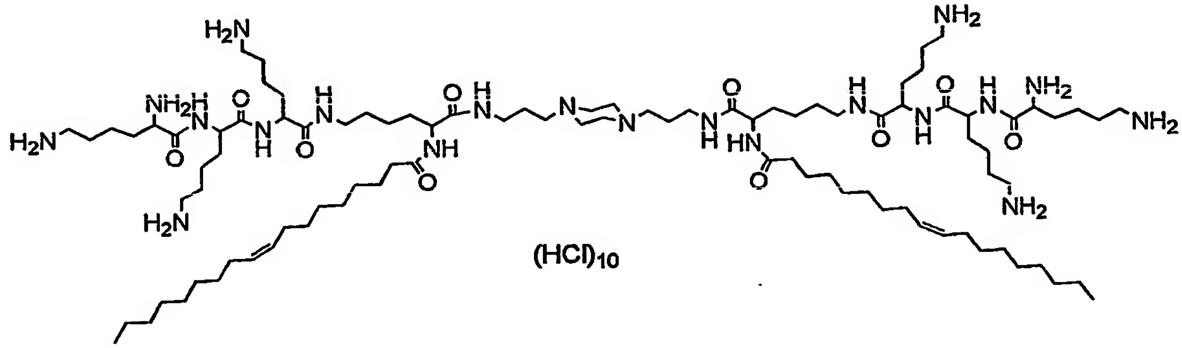
RG 00/797



To a solution of bis aminocompound (140 mg, 0.142 mmol) in THF (48 mL) was added successively a solution of K_2CO_3 (40 mg, mmol, 2.1 eq.) in water (10 mL) and $(Boc)_4$-KKKOSu (256 mg, 0.284 mmol, 2.0 eq.) in THF (10 mL). The reaction was then stirred for 16 h at RT. Most of THF was evaporated and the residue redissolved in $CHCl_3$. Water (10 mL) was added and the organic layer was extracted, washed with 5 % K_2CO_3, water (10 mL) and brine (20 mL). After drying (Na_2SO_4), filtration and evaporation, the residue was purified on SiO_2 (eluent : $CHCl_3$ / MeOH / NEt_3: 85 / 15 / 1, Rf = 0.32). Et_2O is then added and the resulting white solid filtered off. Yield : 0.310 g (0.121 mmol, 85 %). ^{1}H NMR (400 MHz, d_6-DMSO) : δ 8.00 (m, 2 H, 2 NH^{α}), 7.85 (m, 2 H, 2 NH^{α}), 7.75 (m, 4 H, 2 NH^{α} and 2 NHC^{1}), 6.85 (m, 2 H, 2 $N^{\alpha}H$), 6.68 (m, 6 H, 2 x 3 $N^{\varepsilon}H$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.18 (m, 2 H, 2 CH^{α}), 4.09 (m, 4 H, 2 x 2 CH^{α}), 3.82 (m, 4 H, 2 x 2 CH^{α}), 3.00 (m, 6 H, 2 CH_2^{ε} and 2 $CH^{1'}$), 2.75 (m, 2 H, 2 $CH^{1'}$), 2.84 (m, 12 H, 2 x 3 CH_2^{ε}), 2.47 (m, 8 H, 2 x 2 $CH_2^{4'}$), 2.29 (m, 4 H, 2 $CH_2^{3'}$), 2.09 (t, 4 H, J = 9.0, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.65 – 1.15 (m, 168 H), 0.82 (t, 6 H, J = 6.4, 2 CH_3^{18}).

Example 26

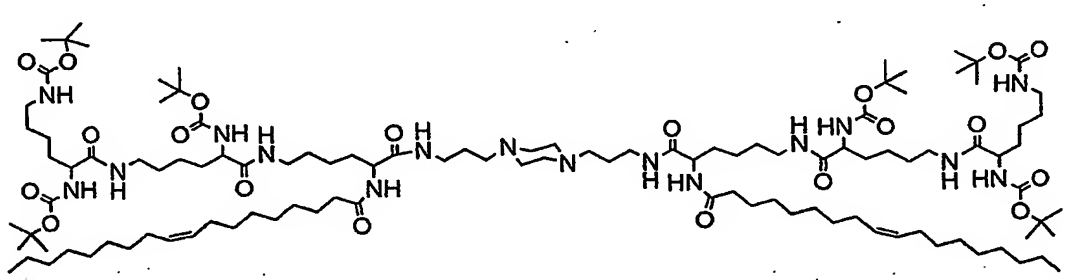
RG 00/805



To a solution of RG00/797 (110 mg, 0.052 mmol) in MeOH (7 mL) was added concentrated HCl (7 mL). The reaction was stirred at RT for 1 h and the solvent were then removed under vaccuum using EtOH to coevaporate. The residue was dissolved in water (40 mL), filtered and evaporated. The compound was dissolved in a minimum amount of MeOH and precipitated with Et_2O. The resulting solid was filtered and collected as a pale brown powder. Yield : 88 mg (0.049 mmol, 94 %). ^{1}H NMR (400 MHz, d_6-DMSO) : δ 8.80 (d, 2 H, J = 7.8, 2 NH^{α}), 8.30 (m, 6 H, 2 x 3 $NH^{\alpha}_{?}$), 8.03 (m, 14 H, 2 NHC^1 and 2 x 3 $N^{\varepsilon}H_3^+$), 5.30 (m, 4 H, 2 $CH^{9,10}$), 4.28 (m, 2 H, 2 CH^{α}), 4.18 (m, 2 H, 2 CH^{α}), 4.08 (m, 2 H, 2 CH^{α}), 3.85 (m, 2 H, 2 CH^{α}), 3.65 (m, 2 H, 2 NH^+), 3.10 (m, 16 H, 2 $CH_2^{\varepsilon 1}$, 2 $CH_2^{3'}$ and 2 x 2 $CH_2^{4'}$), 3.02 (m, 2 H, 2 $CH^{1'}$), 2.95 (m, 2 H, 2 $CH^{1'}$), 2.74 (m, 8 H, 2 x 3 CH_2^{ε}), 2.10 (t, 4 H, J = 7.2, 2 CH_2^2), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.71 (m, 4 H, 2 CH_2^{β}), 1.60 – 1.17 (m, 108 H), 0.84 (t, 6 H, J = 6.4, 2 CH_3^{18}).. MS (+ES) : m/z $[M+H]^{2+}$ 750.1.

Example 27

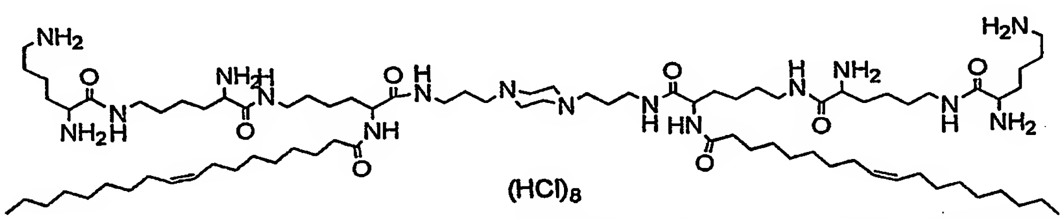
RG 00/823



To a solution of bis aminocompound (76 mg, 0.077 mmol) in THF (40 mL) was added successively a solution of K_2CO_3 (22 mg, 0.159 mmol, 2.06 eq.) in water (2 mL) and Boc_3(K-ε-K)-OSu (105 mg, 0.156 mmol, 2.0 eq.) in THF (8 mL). The reaction was then stirred for 16 h at RT. Most of THF was evaporated and the residue redissolved in $CHCl_3$. Water (10 mL) was added and the organic layer extracted, washed with water (2 x 10 mL) and brine (20 mL). After drying (Na_2SO_4), filtration and evaporation, the residue was purified on SiO_2 (eluent : $CHCl_3$ / MeOH / NEt_3: 91 / 8 / 1, Rf = 0.30). Et_2O is then added and the resulting white solid filtered off. Yield : 0.124 g (0.059 mmol, 77 %). ^1H NMR (400 MHz, d_6-DMSO) : δ 7.79 (m, 4 H, 2 NH^{α} and 2 NH^{ε}), 7.67 (m, 4 H, 2 NHC^1 and 2 NH^{ε}), 6.69 (m, 2 H, 2 NH^{ε}), 8.28 (m, 8 H, 2 x 2 NH^{α}), 5.28 (m, 4 H, 2 $CH^{9,10}$), 4.10 (m, 2 H, 2 $CH^{\alpha 1}$), 3.78 (m, 4 H, 2 x 2 CH^{α}), 3.00 (m, 6 H, 2 CH^{ε} and 2 $CH^{1'}$), 2.98 (m, 2 H, 2 $CH^{1'}$), 3.13 (m, 12 H, 2 x 2 CH_2^{ε}), 2.47 (m, 4 H, 2 $CH_2^{3'}$), 2.25 (m, 8 H, 2 x 2 $CH_2^{4'}$), 2.08 (t, 4 H, J = 7.2, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.80 – 1.14 (m, 138 H), 0.82 (t, 6 H, J = 6.4, 2 CH_3^{18}).

Example 28

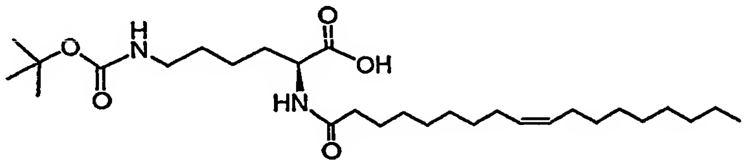
RG 00/830



To a solution of RG00/823 (124 mg, 0.059 mmol) in MeOH (10 mL) was added concentrated HCl (6 mL). The reaction was stirred at RT for 1 h and the solvent were then removed under vaccuum using EtOH to coevaporate. The residue was dissolved in water (40 mL), filtered and evaporated. The compound was dissolved in a minimum amount of MeOH and precipitated with Et_2O. The resulting solid was filtered and collected as a pale pink powder. Yield : 101 mg (0.056 mmol, 96 %). ^1H NMR (400 MHz, d_6-DMSO) : δ 8.73 (t, 2 H, J = 7.8, 2 NH^{ε}), 8.67 (m, 2 H, 2 $NH^{\varepsilon 1}$), 8.28 (m, 8 H, 4 NH_2^{α}), 8.15 (m, 6 H, 2 NHC^1, 2 $NH^{\varepsilon 2}$ and 2 $NH^{\varepsilon 3}$), 7.99 (m, 2 H, 2 $NH^{\alpha 1}$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.09 (m, 2 H, 2 $CH^{\alpha l}$), 3.72 (m, 4 H, 2 $CH^{\alpha 3}$ and 2 $CH^{\alpha 2}$), 3.65 (m, 2 H, 2 $NH^{1'}$), 3.08 (m, 10 H, 2 $CH_2^{\varepsilon 1}$, 2 $CH_2^{\varepsilon 2}$, 2 $CH_2^{3'}$ and 2 $CH_2^{4'}$), 2.74 (m, 2 H, 2 $CH_2^{1'}$), 2.11 (t, 4 H, J = 7.2, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.80 – 1.14 (m, 82 H), 0.83 (t, 6 H, J = 6.4, 2 CH_3^{18}).

Examples 29 to 40 describe alternative routes for the synthesis of GSC170 and derivatives thereof.

Example 29 (RG00/781)



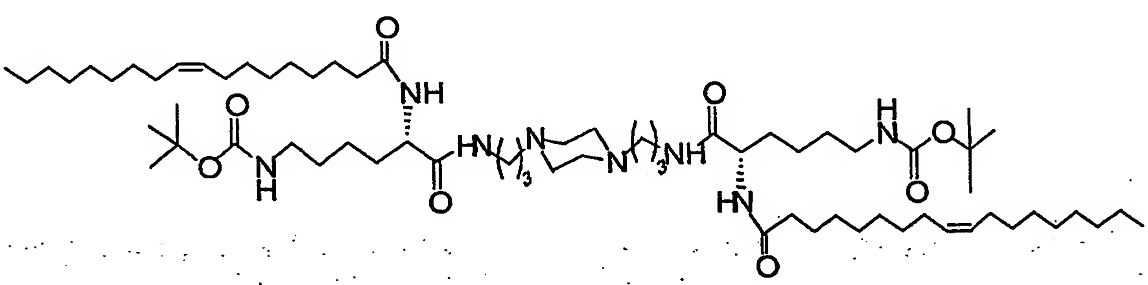
To a solution of N-ε-(t-butoxycarbonyl)-L-lysine (446 mg, 1.89 mmol) in THF (53 mL) were added successively a solution of K_2CO_3 (288 mg, 2.08 mmol) in water (8 mL) and oleoyl succinimidate (754 mg, 1.99 mmol). The reaction was stirred at RT for 15 h and the most of THF was evaporated. Water and $CHCl_3$ (25 mL each) were added and the mixture was acidified with 1 M HCl to pH: 2. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (2 x 20ml). The organic layer was washed with brine (15 mL), dried (Na_2SO_4), filtrated and evaporated to give a white solid. Yield: 940 mg (1.84 mmol, 97%). Rf (SiO_2): 0.28 ($CHCl_3$-MeOH 92:8).

RMN-^{1}H (500 MHz, $CDCl_3$), δ (ppm): 5.33 (m, 2 H, $CH^{9,10}$), 4.15 (m, 1 H, CH^{α}), 3.05 (m, 2 H, CH_2^{ε}), 2.15 (m, 2 H, J = 6.0 Hz, CH_2^{2}), 1.98 (m, 4 H, $CH_2^{8,11}$), 1.80-1.48 (m, 4 H, CH_2^{3}, CH_2^{β}), 1.46-1.20 (m, 33 H, CH_2^{γ}, CH_2^{δ}, $C(CH_3)_3$, 10 CH_2^{ol}), 0.84 (t, 3 H, J = 6.8 Hz, CH_3^{18}).

RMN-^{13}C (125 MHz, $CDCl_3$), δ (ppm): 178.6, 174.4, 156.2, 129.9, 129.7, 78.9, 54.5, 40.0, 36.4, 31.9, 31.3, 29.8, 29.5, 29.4, 29.3, 28.5, 27.3, 27.2, 25.9, 25.0, 22.9, 22.7, 14.1.

HRMS (+ ESI): $C_{29}H_{54}N_2O_5Na$ [M+Na], calcd.: m/z = 533.3930, found: m/z = 533.3903

Example 30 (RG00/518)



To a solution of aminoacid RG00/781 (410 mg, 0.80 mmol) in CH_2Cl_2 (4 ml) were added benzotriazole-1-yl-oxi-tris-pyrrolidino-phosphonium hexafluorphosphate (418 mg, 0.80 mmol), DIEA

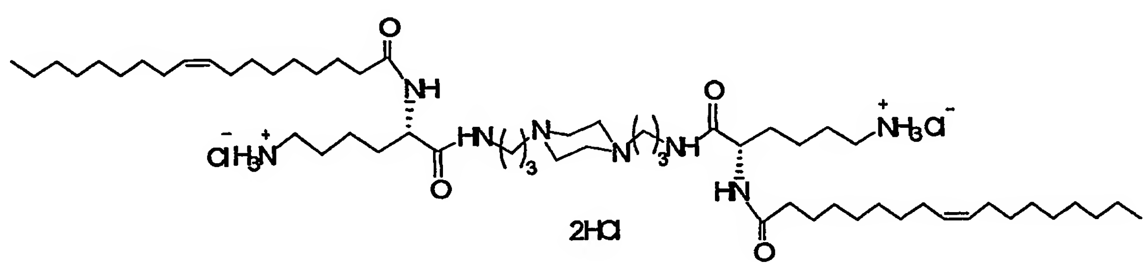
(280 µl, 1.61 mmol) and 1,4-Bis(3-aminopropyl)piperazine (64 µl, 0.31 mmol). The mixture was stirred at RT for 16 h. The solvent was removed, $CHCl_3$ (40mL) was added and the organic layer was washed with 5% $NaHCO_3$ (3 x 12 mL) and brine (30 mL), dried (Na_2SO_4), filtrated and evaporated. The residue was purified by column chromatography on reverse phase (C-18) to give a syrup. Yield: 295 mg (0.25 mmol, 81%), Rf (SiO_2): 0.46 ($CHCl_3$-MeOH 85:15), Rf (C-18): 0.15 (MeOH).

RMN-^{1}H (500 MHz, $CDCl_3$), δ (ppm): 7.49 (m, 2 H, N*H*CO), 6.52 (m, 2 H, N$^{\alpha}$*H*CO), 5.32 (m, 4 H, 2 CH^{9,10}), 4.81 (m, 2 H, N*H*BOC), 4.29 (m, 2 H, CH$^{\alpha}$), 3.30 (m, 4 H, 2 $CH_2^{1'}$), 3.05 (m, 4 H, J = 6.0 Hz, 2 CH_2^{ε}), 2.76 (s broad, 8 H, 4 $CH_2^{4'}$), 2.61 (s broad, H, 2 $CH_2^{3'}$), 2.19 (t, 4 H, J = 7.4 Hz, 2 CH_2^{2}), 1.97 (m, 8 H, 2 $CH_2^{8,11}$), 1.72 (m, 6 H, 2 $CH_2^{2'}$, 2 CH_a^{β}), 1.68 (m, 6 H, 2 CH_2^{3}, 2 CH_b^{β}), 1.52-1.15 (m, 66 H, 2 CH_2^{γ}, 2 CH_2^{δ}, 2 x 10 CH_2^{ol}, 2 $C(CH_3)_3$), 0.85 (t, 6 H, J = 6.7 Hz, 2 CH_3^{18}).

RMN-^{13}C (125 MHz, $CDCl_3$), δ (ppm): 173.6, 172.3, 156.2, 130.0, 129.7, 79.0, 55.7, 53.1, 52.0, 40.0, 38.0, 36.5, 32.0, 31.9, 29.7, 29.5, 29.3, 29.2, 28.4, 27.2, 25.8, 25.7, 24.8, 22.7, 22.6, 14.1.

HRMS (+ ESI): $C_{68}H_{129}N_8O_8$ [M+H], calcd.: m/z = 1185.9928, found: m/z = 1186.0006.

Example 31 (GSC170)



To a solution of RG00/518 (710 mg, 0.60 mmol) in EtOAc (50 mL) was added 4.9 N HCl-EtOAc (50 ml). The reaction was stirred at RT for 2 h. The precipitate was filtrated and washed with ether to give a white solid. Yield 542 mg (0.48 mmol, 80%), Rf (SiO_2): 0.63 (MeOH-NH_4OH 80:20).

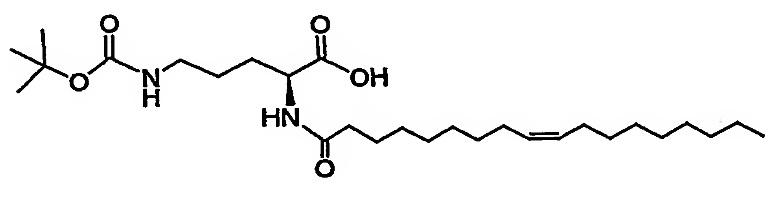
RMN-^{1}H (500 MHz, DMSO-d_6), δ (ppm): 8.12 (m, 2 H, N*H*CO), 8.05-7.87 (m, 8 H, N$^{\alpha}$*H*CO, 2 N$^{\varepsilon}H_3^+$), 5.31 (m, 4 H, 2 CH^{9,10}), 4.10 (m, 2 H, J = 5.0 Hz, 8.4 Hz, 2 CH$^{\alpha}$), 3.70 (s broad, 4 H, 4 CH $_{axial}$ $^{4'}$), 3.44 (s broad, 4 H, 4 CH$_{ecuat.}$ $^{4'}$), 3.10 (m, 8 H, 2 $CH_2^{1'}$, 2 $CH_2^{3'}$), 2.72 (m, 4 H, J = 6.2 Hz, 2 CH_2^{ε}), 2.12 (m, 4 H, J = 6.8 Hz, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,11}$), 1.84 (m, 4 H, J = 6.3 Hz, 2 $CH_2^{2'}$), 1.65-1.40 (m, 12 H, 2 CH_2^{β}, 2 CH_2^{δ}, 2 CH_2^{3}), 1.35-1.15 (m, 44 H, 2 CH_2^{γ}, 2 x 10 CH_2^{ol}), 0.83 (t, 6

H, J = 6.7 Hz, 2 CH_3^{18}).

RMN-^{13}C (125 MHz, DMSO-d_6), δ (ppm): 172.5, 172.1, 129.7, 55.0, 53.7, 52.7, 48.1, 40.1, 39.9, 39.8, 39.6, 39.4, 39.3, 39.1, 38.5, 31.4, 29.3, 29.2, 28.9, 28.8, 28.7, 28.7, 26.7, 25.3, 22.2, 14.1.

HRMS (+ ESI): $C_{58}H_{113}N_8O_4$ [M+H], calcd.: m/z = 985.8879, found: m/z = 985.8805.

Example 32 (Compound 4)



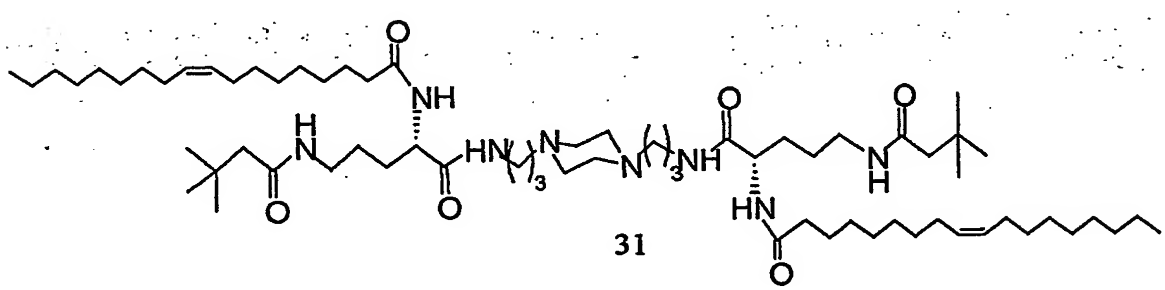
To a solution of N-δ-(t-butoxycarbonyl)-L-ornitine (440 mg, 1.89 mmol) in THF (53 mL) were added successively a solution of K_2CO_3 (288 mg, 2.08 mmol) in water (8 mL) and oleoyl succinimidate (755 mg, 1.99 mmol). The reaction was stirred at RT for 15 h and the most of THF was evaporated. Water and $CHCl_3$ (25 mL each) were added and the mixture was acidified with 1 M HCl to pH: 2. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (2 x 20ml). The organic layer was washed with brine (15 mL), dried (Na_2SO_4), filtrated and evaporated to give a white solid. Yield: 898 mg (1.81 mmol, 96%). Rf (SiO_2): 0.16 ($CHCl_3$-MeOH 92:8).

RMN-^{1}H (500 MHz, $CDCl_3$), δ (ppm): 7.03 (m, 1 H, N*H*CO), 5.32 (m, 2 H, $CH^{9,10}$), 4.40 (m, 1 H, CH^{α}), 3.11 (m, 2 H, CH_2^{δ}), 2.19 (t, 2 H, J = 7.4 Hz, CH_2^{2}), 1.99 (m, 4 H, $CH_2^{8,11}$), 1.85 (m, 1 H, CH_a^{β}), 1.70-1.20 (m, 34 H, CH_b^{β}, CH_2^{3}, CH_2^{γ}, $C(CH_3)_3$, 10 CH_2^{ol}), 0.87 (t, 6 H, J = 6.7 Hz, CH_3^{18}).

RMN-^{13}C (125 MHz, $CDCl_3$), δ (ppm): 176.6, 174.4, 156.5, 130.0, 129.7, 79.4, 53.0, 39.8, 36.4, 33.9, 31.9, 29.8, 29.5, 29.4, 29.3, 29.1, 28.9, 28.4, 27.2, 26.4, 25.7, 25.6, 24.8, 22.7, 14.1.

HRMS (+ ESI): $C_{28}H_{52}N_2O_2$ [M+H], calcd.: m/z = 519.3774, found: m/z = 519.3767.

Example 33 (Compound 5)



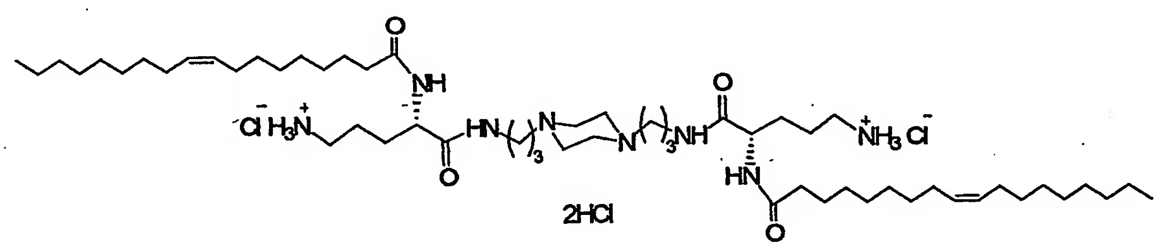
To a solution of aminoacid 4 (548 mg, 1.10 mmol) in CH_2Cl_2 (5.5 ml) were added benzotriazole-1-yl-oxi-tris-pyrrolidino-phosphonium hexafluorphosphate (574 mg, 1.10 mmol), DIEA (385 µl, 2.20 mmol) and 1,4-Bis(3-aminopropyl)piperazine (84 µl, 0.41 mmol). The mixture was stirred at RT for 18 h. The solvent was removed, $CHCl_3$ (40mL) was added and the organic layer was washed with 5% $NaHCO_3$ (3 x 10 mL) and brine (25 mL), dried (Na_2SO_4), filtrated and evaporated. The residue was purified by column chromatography on reverse phase (C-18) to give a syrup. Yield: 360 mg (0.31 mmol, 76%), Rf (SiO_2): 0.46 ($CHCl_3$-MeOH 85:15), Rf (C-18): 0.22 (MeOH).

RMN-^{1}H (500 MHz, $CDCl_3$), δ (ppm): 7.60 (m, 2 H, 2 N*H*CO), 6.50 (m, 2 H, J = 6.5 Hz, 2 $N^{\alpha}HCO$), 5.36 (m, 4 H, 2 $CH^{9,10}$), 4.85 (m, 2 H, 2 N*H*BOC), 4.48 (m, J = 5.7 Hz, 2 H, 2 CH^{α}), 3.38 (m, J = 13.2 Hz, J = 6.6 Hz, 2 H, 2 $CH_a^{1'}$), 3.28 (m, 4 H, 2 $CH_b^{1'}$, 2 CH_a^{δ}), 3.11 (m, 2 H, J = 13.2 Hz, J = 6.3 Hz, 2 CH_b^{δ}), 2.48 (m, 12 H, 2 $CH_2^{3'}$, 4 $CH_2^{4'}$), 2.23 (t, 4 H, J = 7.8 Hz, 2 CH_2^{2}), 2.03 (m, 8 H, 2 $CH_2^{8,11}$), 1.87-1.40 (m, 34 H, 2 CH_2^{β}, 2 $CH_2^{2'}$, 2 CH_2^{3}, 2 CH_2^{γ}, 2 $C(CH_3)_3$), 1.30-1.22 (m, 40 H, 2 x 10 CH_2^{cl}), 0.91 (t, 6 H, J = 6.4 Hz, 2 CH_3^{18}).

RMN-^{13}C (125 MHz, $CDCl_3$), δ (ppm): 173.2, 171.5, 156.4, 130.0, 129.8, 79.2, 57.0, 53.3, 52.2, 39.7, 39.2, 36.7, 31.9, 30.6, 29.8, 29.5, 29.3, 29.2, 28.5, 27.2, 26.5, 25.7, 25.2, 22.7, 14.1.

HRMS (+ ESI): $C_{58}H_{113}N_8O_4$ [M+H], calcd.: m/z 1157.9615, found: m/z = 1157.9531.

Example 34 (GSC170 Orn)



To a solution of compound 5 (138 mg, 0.12 mmol) in EtOAc (10 mL) was added 4.9 N HCl-EtOAc (10 ml). The reaction was stirred at RT for 2 h. The precipitate was filtrated and washed with ether to give a white solid. Yield 116 mg (0.10 mmol, 83%), Rf (SiO_2): 0.61 (MeOH-NH_4OH 80:20).

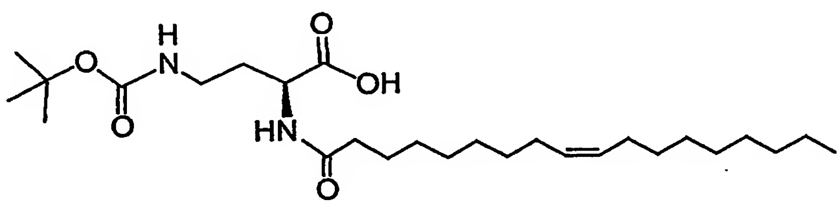
RMN-^{1}H (500 MHz, $CDCl_3$), δ (ppm): 8.24 (s broad, 2 H, 2 N*H*CO), 8.04 (d, 2 H, J = 7.8 Hz, 2

$NH^{\alpha}CO$), 7.94 (s broad, 6 H, 2 NH_3^+), 5.33 (m, 4 H, 2 $CH^{9,10}$), 4.20 (m, J = 5.5 Hz, 2 H, 2 CH^{α}), 3.76 (m, 4 H, 4 $CH_{axial}^{4'}$), 3.48 (m, 4 H, 4 $CH_{ecuat}^{4'}$), 3.12 (m, 8 H, 2 $CH_2^{1'}$, 2 $CH_2^{3'}$), 2.76 (m, 4 H, J = 5.7 Hz, 2 CH_2^{δ}), 2.14 (t, 4 H, J = 7.4 Hz, 2 CH_2^{2}), 1.98 (m, 8 H, 2 $CH_2^{8,11}$), 1.84 (m, 4 H, 2 $CH_2^{2'}$), 1.70 (m, 2 H, 2 CH_a^{β}), 1.64-1.40 (m, 10 H, 2 CH_b^{β}, 2 CH_2^{3}, 2 CH_2^{γ}), 1.24 (m, 40 H, 2 x 10 CH_2^{ol}), 0.86 (t, 6 H, J = 6.7 Hz, 2 CH_3^{18}).

RMN-^{13}C (125 MHz, $CDCl_3$), δ (ppm): 172.5, 171.8, 129.7, 79.3, 53.8, 51.9, 48.1, 38.3, 35.3, 31.4, 29.3, 29.2, 28.9, 28.8, 28.7, 26.7, 25.3, 22.2, 14.1.

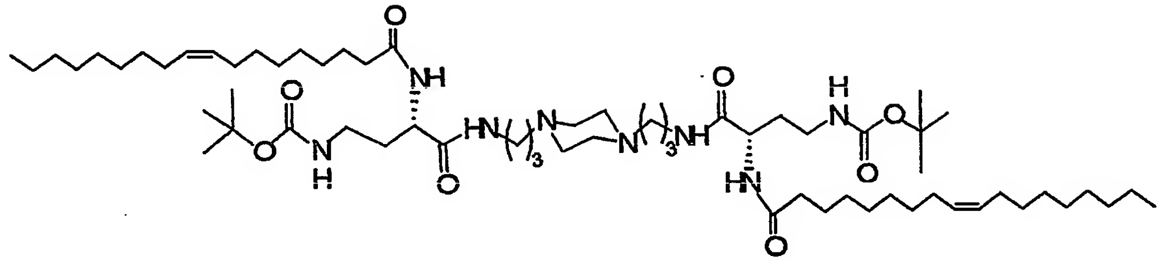
HRMS (+ ESI): $C_{58}H_{113}N_8O_4$ [M+H], calcd.: m/z 957.8572, found: m/z = 957.8575.

Example 35 (Compound 7)



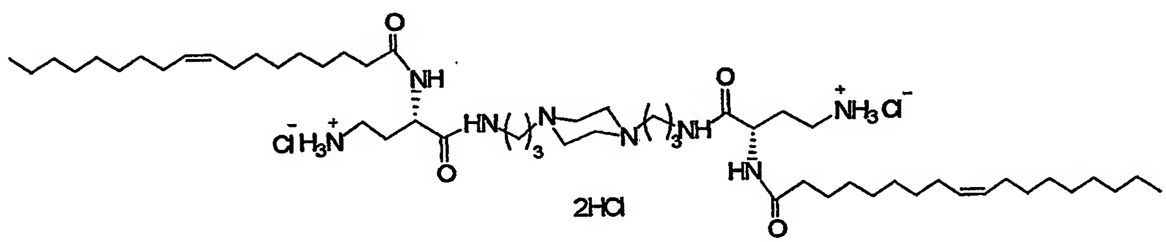
To a solution of N-γ-(t-butoxycarbonyl)-L-diaminobutyric acid (203 mg, 0.93 mmol) in THF (27 mL) were added successively a solution of K_2CO_3 (141 mg, 1.02 mmol) in water (4 mL) and oleoyl succinimidate (371 mg, 0.98 mmol). The reaction was stirred at RT for 16 h and the most of THF was evaporated. Water and $CHCl_3$ (10 mL each) were added and the mixture was acidified with 1 M HCl to pH: 2. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (2 x 10ml). The organic layer was washed with brine (8 mL), dried (Na_2SO_4), filtrated and evaporated to give a syrup. Yield: 441 mg (0.91 mmol, 98%). Rf (SiO_2): 0.35 ($CHCl_3$-MeOH 85:15).

Example 36 (Compound 8)



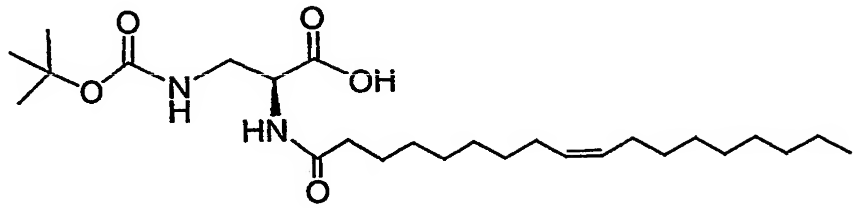
To a solution of aminoacid 7 (410 mg, 0.85 mmol) in CH_2Cl_2 (4 ml) were added benzotriazole-1-yl-oxi-tris-pyrrolidino-phosphonium hexafluorphosphate (442 mg, 0.85 mmol), DIEA (296 µl, 1.70 mmol) and 1,4-Bis(3-aminopropyl)piperazine (67 µl, 0.33 mmol). The mixture was stirred at RT for 16 h. The solvent was removed, $CHCl_3$ (40mL) was added and the organic layer was washed with 5% $NaHCO_3$ (3 x 12 mL) and brine (30 mL), dried (Na_2SO_4), filtrated and evaporated. The residue was purified by column chromatography on reverse phase (C-18) to give a syrup. Yield: 316 mg (0.28 mmol, 85%), Rf (SiO_2): 0.51 ($CHCl_3$-MeOH 85:15), Rf (C-18): 0.14 (MeOH).

Example 37 (GSC170 Dab)



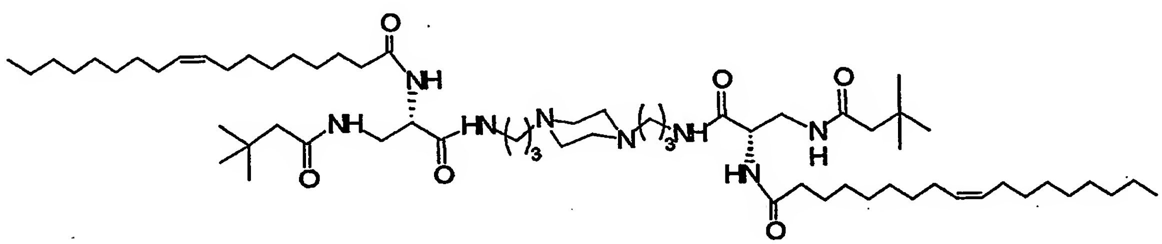
To a solution of compound 8 (244 mg, 0.22 mmol) in EtOAc (15 mL) was added 4.9 N HCl-EtOAc (15 ml). The reaction was stirred at RT for 2 h. The precipitate was filtrated and washed with ether to give a white solid. Yield 198 mg (0.18 mmol, 82%), Rf (SiO_2): 0.52 (MeOH-NH_4OH 85:15).

Example 38 (Compound 10)



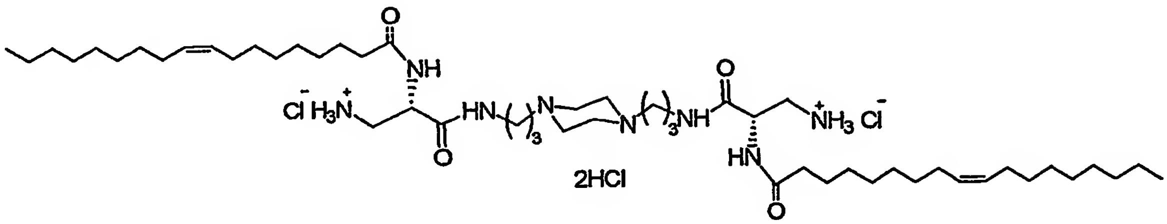
To a solution of N-β-(t-butoxycarbonyl)-L-diaminopropionic acid (560 mg, 2.74 mmol) in THF (77 mL) were added successively a solution of K_2CO_3 (416 mg, 3.02 mmol) in water (11 mL) and oleoyl succinimidate (1.04 g, 2.74 mmol). The reaction was stirred at RT for 18 h and the most of THF was evaporated. Water and $CHCl_3$ (30 mL each) were added and the mixture was acidified with 1 M HCl to pH: 2. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (2 x 30ml). The organic layer was washed with brine (20 mL), dried (Na_2SO_4), filtrated and evaporated to give a white solid. Yield: 1.25 g (2.67 mmol, 97%). Rf (SiO_2): 0.35 ($CHCl_3$-MeOH 85:15).

Example 39 (Compound 11)



To a solution of aminoacid **10** (1.22 g, 2.60 mmol) in CH_2Cl_2 (14 ml) were added benzotriazole-1-yl-oxi-tris-pyrrolidino-phosphonium hexafluorphosphate (1.35 g, 2.60 mmol), DIEA (910 µl, 5.21 mmol) and 1,4-Bis(3-aminopropyl)piperazine (206 µl, 1.00 mmol). The mixture was stirred at RT for 15 h. The solvent was removed, $CHCl_3$ (75mL) was added and the organic layer was washed with 5% $NaHCO_3$ (3 x 30 mL) and brine (60 mL), dried (Na_2SO_4), filtrated and evaporated. The residue was purified by column chromatography on reverse phase (C-18) to give a syrup. Yield: 940 mg (0.85 mmol, 85%), Rf (SiO_2): 0.52 ($CHCl_3$-MeOH 85:15), Rf (C-18): 0.16 (MeOH).

Example 40 (GSC170 Dap)



To a solution of compound 11 (355 mg, 0.32 mmol) in EtOAc (30 mL) was added 4.9 N HCl-EtOAc (30 ml). The reaction was stirred at RT for 2 h. The precipitate was filtrated and washed with ether to give a white solid. Yield 280 mg (0.27 mmol, 84%), Rf (SiO_2): 0.34 (MeOH-NH_4OH 99:1).

Example 41 Transfection of recombinant plasmid expressing luciferase into cells using lysine-polyamine-based gemini compounds.

Transfection of recombinant plasmid expressing luciferase into cells using lysine-polyamine-based gemini compounds. Transfection studies were performed using the adherent cell line CHO-DG44. Complete medium consisted of MEM alpha medium supplemented with 10 % v/v foetal bovine serum and 1x L-Glutamine. All media and supplements were obtained from Life Technologies. In Vitro Gene Transfection. Cells were seeded into Biocat poly-D-lysine 96-well black plates (BD) 16-18 hours prior to transfection at an approximate density of 3 x 104 cells per well. For transfection, 0.1 mg of the luciferase reporter gene plasmid, pGL3-Control Vector (Promega) per well, was incubated with various concentrations of the diaminoacid-polyamine:peptide-based gemini compounds and complexing agents in a final volume of 100 µl. After 30 minutes incubation at RT, OPTI-MEM® medium (Life Technologies) was added to the transfection mixture and the solution placed on the cells (final volume per well: 100 µl). Following a 3 hour or over night incubation at 37°C, the transfection solution was replaced with complete medium and the cells incubated further at 37*C. Reporter gene assays were performed according to the manufacturer's guidelines (Roche Diagnostics) approximately 48 hours post transfection. Luminescence was measured in a Packard TopCount NXT Microplate Scintillation and Luminescence Counter. Figure 4. Transfection of CHO-DG44 cells with Gemini surfactant

GSC102. The numbers along the x-axis refer to concentration of gemini compounds in mM. The block of 5 bars at the right of the chart shows the data obtained when DNA was premixed with poly-lysine. The block of 5 bars at the left side shows data when no poly-lysine is used. The figures on the Y-axis represent CPS (count per second) from the luciferase assay. Bars represent the mean CPS of 4 experiments ± the standard error of the mean. Figure 5. Transfection of CHO-DG44 cells with Gemini surfactant GSN14. Bars represent the mean CPS (counts per second) of 4 experiments ± the standard error of the mean. Figure 6. Transfection of CHO-DG44 cells with Gemini surfactant GSC197. Bars represent the mean CPS (counts per second) of 4 experiments ± the standard error of the mean.

Example 42

Delivery of fluorescent oligonucleotides to cell lines/primary cells using Gemini Surfactant 170 (GSC170)

GSC170 (1 mg/ml in water) was diluted to a 10x solution with Optimem serum free media. A FITC-tagged oligonucleotide was similarly diluted in Optimem at 10x final concentration. The GSC170 and oligonucleotide were then mixed 1:1 and incubated for fifteen minutes at room temperature. The adherent cell lines: RBL-2H3, J774 and 16HBE14o were plated out the day before transfection. Murine primary T cells were transfected either inactivated or after differentiation into T helper 2 cells. GSC170:oligo complexes were diluted to 1x in Optimem and added to adherent cells that had been washed once in Optimem then all media removed. Nuclear delivery of the oligonucleotide was oserved over a period of 24 hours and compared to the commercial reagent, Lipofectamine 2000 (LF2K).

Transfection efficiencies are shown in Table 1:

Table 1 – Transfection efficiencies using GSC170

	GSC170 (%nuclear)	LF2K (%nuclear)
RBL-2H3	50%	#
J774	50%	50%
16HBE14o	50%	30%
Primary inactivated T cells	60%	#
Activated T helper 2 cells	60%	#

= too low to estimate

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

Brief description of the drawings

Figure 1. General scheme for synthesis of diaminoacid-polyamine:peptide based gemini compounds wherein the hydrophobic tail is linked to the α-amino group of a diaminoacid further linked to a polyamine moiety by amide bonds.

Figure 2. General scheme for synthesis of diaminoacid-polyamine:peptide based gemini compounds wherein the hydrophobic tail is linked to the terminalamino group of a diaminoacid further linked to a polyamine moiety by amide bonds.

Figure 3. General scheme for the synthesis of diaminoacid-aminoacid-polyamine:peptide based gemini compounds wherein an aminoacid is linked by an amide bond to the α-amino group of a diaminoacid further linked to a polyamine moiety by amide bonds.

Figure 4. Transfection of recombinant plasmid expressing luciferase into CHO-DG44 cells using GSC102. The numbers along the x-axis refer to concentration of the gemini compound in mM. The block of 5 bars at the right of the chart shows the data obtained when DNA was premixed with poly-lysine. The block of 5 bars at the left side shows data when no poly-lysine is used. The figures on the Y-axis represent CPS (count per second) from the luciferase assay. Bars represent the mean CPS of 4 experiments ± the standard error of the mean.

Figure 5. Transfection of recombinant plasmid expressing luciferase into CHO-DG44 cells using GSN14. The numbers along the x-axis refer to concentration of the gemini compound in mM. The block of 5 bars at the right of the chart shows the data obtained when DNA was premixed with poly-lysine. The block of 5 bars at the left side shows data when no poly-lysine is used. The figures on the Y-axis represent CPS (count per second) from the luciferase assay. Bars represent the mean CPS of 4 experiments ± the standard error of the mean.

Figure 6. Transfection of recombinant plasmid expressing luciferase into CHO-DG44 cells using GSC197. The numbers along the x-axis refer to concentration of the gemini compound in mM. The block of 5 bars at the right of the chart shows the data obtained when DNA was premixed with poly-lysine. The block of 5 bars at the left side shows data when no poly-lysine is used. The figures on the

Y-axis represent CPS (count per second) from the luciferase assay. Bars represent the mean CPS of 4 experiments ± the standard error of the mean.